SERUM HOMOCYSTEINE, VITAMIN B_{12} AND FOLATE IN GHANAIAN WOMEN WITH HYPERTENSIVE DISORDERS OF PREGNANCY

A THESIS SUBMITTED IN FULFILLMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF PHILOSOPHY

In the

Department of Molecular Medicine,

School of Medical Sciences

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NOVEMBER, 2016.

DECLARATION

The research work described in this thesis was carried out at the Department of Molecular Medicine, School of Medical Sciences, KNUST. This work has not been submitted for any other degree. Information taken from other works has been specially and duly acknowledged.

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ABSTRACT

Hypertensive disorders of pregnancy are common complication occurring during pregnancy, and are associated with maternal and fetal mortality and morbidity. Hyperhomocysteinaemia, a known risk factor for vascular disease, could play a significant role in the aetiopathogenesis of pregnancy-induced hypertension (PIH). This study, therefore, evaluated the maternal serum concentrations of homocysteine, vitamin B_{12} and folate in normal pregnancy (NP) and pregnant women presenting with preeclampsia (PE) and gestational hypertension (GH). This randomized case-control study involved 30 PE patients, 30 GH patients and 30 age-matched normotensive uncomplicated pregnant women (control group) in the third trimester of pregnancy. After obtaining an informed consent from each participant, information on socio-demographic characteristics, medical history and previous obstetric history was obtained. Blood pressure, anthropometric measurements and blood sample were taken for the estimation of homocysteine, vitamin B_{12}, folate and lipid profile of each woman. Mean levels of maternal serum homocysteine was significantly higher in PIH, PE and GH patients when compared with NP women ($p<0.05$). Although mean vitamin B_{12} and folate were decreased in the PIH, PE and GH patients when compared with the normal pregnant women, it was only in the PIH and the PE patients that the differences were significant ($p<0.05$). In the PIH patients, there was a statistically significant negative correlation between homocysteine and folate ($r=-0.283$, $p<0.05$). While none of the normal pregnant women had intrauterine growth restriction (IUGR) or low birthweight (LBW), thirty-five percent (35%) and twenty-eight percent (28%) of the participants with PIH demonstrated IUGR and LBW respectively. Except for the GH patients where estimated foetal weight (EFW) was insignificantly lower, EFW and birthweight were significantly lower in the PIH (PE and GH) patients when compared with the NP women. The use of the contraceptive Depo-Provera prior to pregnancy was significantly associated with about thirty-fold (30) increase in the odds of developing preeclampsia ($OR=29.71$, $p<0.001$). There was a significant ($p<0.01$) positive correlation between homocysteine and blood pressure (systolic and diastolic blood pressure) in the PIH patients. Maternal serum concentration of homocysteine is altered in PIH (PE and GH) when compared with normal pregnancy, and this imbalance is depicted by an elevated serum concentration of homocysteine with a correspondingly decreased serum concentrations of vitamin B_{12} and folate. Hyperhomocysteinaemia in pregnancy could play a significant role in the aetiopathogenesis of pregnancy induced hypertension, intrauterine growth restriction and low birthweight. Furthermore, the use of the contraceptive Depo-Provera by women prior to pregnancy predisposes them to a high risk of developing preeclampsia.

ACKNOWLEDGEMENT

My deepest gratitude goes to God Almighty from whom all good things come for His grace and mercy towards me and for seeing me through the execution of this programme. Without Him, this programme will not have been successful.

I extend my sincere gratitude to my supervisors, Dr. W. K. B. A. Owiredu and Dr. Christian Obirikorang of the Department of Molecular Medicine, KNUST as well as Prof. George Asare of School of Allied Health Science, University of Ghana for their countless pieces of advice, corrections, explanations and words of encouragement throughout this programme.

I am also grateful to Dr. Leila Seidu, Dr. Cham Momodou and Mrs. Veronica Parkoo of Comboni Hospital for their technical support. My sincere thanks to all the pregnant women who made this study a reality.

Special thanks to Mr. Worlanyo Tashie of Ho Municipal Hospital for contribution in diverse ways. I also humbly acknowledge the entire staff of the laboratory, antenatal care and maternity units of the Comboni Hospital for their technical and moral support during the research work.

Finally, I wish to express my sincere thanks to all my loved ones and family members for their support and prayers.

God richly bless you all.

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ABBREVIATIONS

3, 5-DHBS	3, 5-dichloro-2-hydroxybenzene
4-AAP	4-aminoantipyrine
ADP	Adenosine-5-diphosphate
ANC	Antenatal care
ATP	Adenosine triphosphate
BHMT	Betaine-homocysteine methyl-transferase
BMI	Body mass index
BWT	Birthweight
CBS	Cystathionine β-synthase
CI	Confidence interval
COC	combined oral contraceptive
DAP	Dihydroxyacetone Phosphate
DBP	Diastolic blood pressure
DNA	Deoxyribonucleic acid
EFW	Estimated foetal weight
ELISA	Enzyme- Linked Immunosorbent Assay
ET-1	Endothelin-1

FMD	Flow-mediated dilation
G3P	Glycerol -3- phosphate
GH	Gestational Hypertension
GK	Glycerol Kinase
GPO	Glycerophosphate Oxidase
Hcy	Homocysteine
HDL	High Density Lipoprotein
HDL-C	High Density Lipoprotein Cholesterol
HLA-C	Human leukocyte antigen C
IUGR	Intrauterine growth restriction
KIRs	Killer immunoglobulin receptors
LBW	Low birth weight
LDH	Lactate dehydrogenase
LDL	Low Density Lipoprotein
LDL-C	Low Density Lipoprotein Cholesterol
MCP-1	Monocyte chemo-attractant protein 1
MDA	Malondialdehyde
MS	methionine synthase

MTHFR	Methelenetetrahydrofolate reductase
NK cells	Natural killer cells
NO	Nitric oxide
NOS	Nitric oxide synthase
OR	Odds Ratio
PE	Preeclampsia
PIH	Pregnancy Induced Hypertension
PLGF	Placental growth factor
ROS	Reactive oxygen species
SAH	S-adenosyl homocysteine
SAM	S-adenosyl methionine
SBP	Systolic blood pressure
sEng	Soluble Endoglin
sFlt-1	Soluble Foetal-like tyrosine kinase 1
SGA	Small for gestational age
TC	Total Cholesterol
TCEP	Tris [2-carboxyethyl] phosphine
TG	Triglycerides
TG	Triglycerides

TGFβ	Transforming growth factor β
tHcy	Total homocysteine
USA	United States of America
VEGF	Vascular endothelial growth factor
VEGFR-1	Vascular endothelial growth factor receptor 1
VLDL	Very Low density Lipoprotein

Chapter 1

INTRODUCTION

1.1 BACKGROUND

Hypertensive disorders are among the common complications during pregnancy and contributes significantly to maternal and perinatal morbidity and mortality worldwide Leveno (2013). Pregnancy-induced hypertension (PIH) is a generic term used to define significant rise in blood pressure (systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg) during pregnancy, occurring after 20 weeks of gestation in a woman without prior hypertension. When accompanied by significant proteinuria, the disorder is termed preeclampsia and when it is without significant proteinuria, it is termed gestational hypertension (Leeman & Fontaine, 2008; Leveno, 2013; Owiredu *et al.*, 2012).

Gestational hypertension is generally characterized by good maternal and foetal outcomes. Gestational hypertension is referred to as transient hypertension if preeclampsia does not develop and the blood pressure has returned to normal by 12 weeks postpartum. Importantly, women with gestational hypertension may develop other signs associated with preeclampsia - for example, headaches, epigastric pain, or thrombocytopenia - which influences management (Leeman & Fontaine, 2008; Leveno, 2013).

Although pathophysiology of preeclampsia is poorly understood, endothelial dysfunction is most popularly hypothesized to be a central pathophysiological feature of preeclampsia leading to altered vascular reactivity, loss of vascular integrity and activation of the coagulation cascade (Sangeeta *et al.*, 2013; Var *et al.*, 2003).

The incidence of preeclampsia is commonly cited to be about 5% although remarkable variations are reported (Leveno, 2013). The incidence is influenced by parity, with nulliparous women having

a greater risk when compared with multiparous women. Other risk factors associated with preeclampsia include multiple pregnancy, history of chronic hypertension, maternal age over 35 years, excessive maternal weight (Leveno, 2013). Intrauterine growth restriction (IUGR), preterm delivery, low birth weight, foetal death and neonatal death due to complications of pre-term delivery are common perinatal outcomes associated with pregnancy-induced hypertension (Lehrer *et al.*, 1993; Leveno, 2013; Mahal *et al.*, 2009).

Elevated serum homocysteine has been claimed as a risk factor for vascular endothelial cell injury in preeclampsia and its consequences (Mahal *et al.*, 2009). Experimental studies revealed that moderately elevated homocysteine concentrations may induce cytotoxic and oxidative stress, leading to endothelial cell impairment. Additionally, exposure of trophoblast cells to homocysteine (20 µmol/L) may increase cellular apoptosis and lead to inhibition of trophoblastic function (Bergen *et al.*, 2012; Mahal *et al.*, 2009)

Homocysteine (Hcy), a sulfur-containing amino acid, is formed by the demethylation of the essential amino acid methionine. It can be recycled into methionine or converted into cysteine with the aid of B-vitamins. Elevated serum homocysteine beyond the normal reference range (515 µmol/L) is traditionally referred as hyperhomocysteinaemia. Hyperhomocysteinaemia is further subcategorized into moderate (15-30 µmol/L), intermediate (30-100 µmol/L), and severe (>100) µmol/L (Ciaccio *et al.*, 2008; Selhub & Mayer, 1999). High serum homocysteine levels could result from a genetic defect in enzymes involved in homocysteine metabolisms (defects in cystathionine β synthase, methionine synthase, or methelenetetrahydrofolate reductase); nutritional deficiency in vitamins (vitamins B_6, vitamins B_{12} and folate), renal failure for effective amino acid clearance and drug interactions (Lawrence-de-Koning *et al.*, 2003).

The mean homocysteine levels normally decrease with gestation either due to physiological response to the pregnancy, increase in estrogen, hemodilution from increased plasma volume or increased demand for methionine by both the mother and fetus. The levels are the lowest during second trimester of pregnancy and increase in the second half of the third trimester of pregnancy (Mahal *et al.*, 2009; Mukhopadhyay *et al.*, 2014). Dyslipidemia also plays a role in the aetiopathogenesis of pregnancy-induced hypertension. Human gestation is associated with an atherogenic lipid profile that is further enhanced in preeclampsia. Such profile may also be a potential contributor to endothelial cell dysfunction, which is a central feature in the pathophysiology of preeclampsia (Mahal *et al.*, 2009).

Some studies have indicated that the complications of preeclampsia are low birth weight, intrauterine growth restriction (IUGR) and fetal loss (Ghike *et al.*, 2011; Mukhopadhyay *et al.*, 2014). However, data on such study in Ghana and the sub-Saharan region remain scarce.

1.2 PROBLEM STATEMENT

Despite the numerous strategies devised by the international community to curb maternal mortality, it still remains a major Public Health challenge (UN, 2009). Globally, maternal mortality is the leading cause of death among females aged 15-49 years old. More than 1500 women die each day from pregnancy related causes resulting in an estimated 550 000 maternal deaths annually (UN, 2009). Preeclampsia is a pregnancy specific disorder, which complicates 710% of all gestations. Approximately 10-15% of maternal deaths in developing countries are associated with preeclampsia (Mahal *et al.*, 2009). Pregnancy-induced hypertension causes a number of problems, including intrauterine growth restriction, fetal loss, and low birth weight, for both mother and baby (Ghike *et al.*, 2011; Leeman & Fontaine, 2008; Sangeeta *et al.*, 2013). Some studies have linked maternal homocysteine levels to pregnancy-induced hypertension and pregnancy outcomes such

as intrauterine growth restriction, fetal loss, and low birth weight (Ghike *et al.*, 2011; Mukhopadhyay *et al.*, 2014). However, evidence on this is conflicting with some studies stating that serum homocysteine values have no correlation to maternal and fetal outcome (Infante-Rivard *et al.*, 2003). Also, data on this in sub-Saharan Africa remain scarce.

1.3 JUSTIFICATION

Hyperhomocysteinemia, a known risk factor for vascular disease, was incriminated as one of the predisposing risk factors for pregnancy-induced hypertension (Ghike *et al.*, 2011; Mukhopadhyay *et al.*, 2014). Serum homocysteine levels were found to be significantly high in preeclamptic patients, with several studies relating hyperhomocysteinaemia to preeclampsia and other adverse pregnancy outcomes such as IUGR and low birth weight (Ghike *et al.*, 2011; Mukhopadhyay *et al.*, 2014). If this study establishes a significant association between maternal homocysteine levels and pregnancy complications such as preeclampsia, IUGR and low birth weight, then maternal serum homocysteine could be a predictive marker well ahead of blood pressure changes and ultimately provide scope for prevention and treatment by supplementation of B_{12} and folic acid.

1.4 AIM

The aim of this study therefore was to investigate maternal concentrations of serum homocysteine, vitamin B_{12}, folate and lipids in pregnancies complicated by pregnancy-induced hypertension and to determine whether these parameters were associated with intrauterine growth restriction (IUGR) and low birth weight.

1.5 OBJECTIVE

Specifically, this study sought:

1. To assess the serum concentrations of homocysteine, vitamin B_{12}, folate and lipid profile in pregnancy induced hypertension (gestational hypertension and preeclampsia) and normal uncomplicated pregnancies.
2. To determine the IUGR and infant body weight in study participants.
3. To determine the correlation between the studied parameters.

1.6 HYPOTHESIS

Elevated serum homocysteine concentration is significantly correlated with pregnancy-induced hypertension, intrauterine growth restriction and infant body weight.

Chapter 2

LITERATURE REVIEW

2.1 HYPERTENSIVE DISORDERS IN PREGNANCY

Hypertensive disorders are one of the commonest medical disorders encountered in pregnancy and remains an important cause of maternal and perinatal morbidity and mortality worldwide. Hypertension in pregnancy is defined as a systolic blood pressure of 140 mm Hg or greater or a diastolic blood pressure of 90 mm Hg or greater measured two times with at least a 6-hour interval (Mustafa *et al.*, 2012). Hypertension is a sign of an underlying pathology which may be preexisting or appears for the first time during pregnancy. The identification of this clinical entity and effective management play a significant role in the outcome of pregnancy, both for the mother and the baby. In developing countries with inadequately cared pregnancy, this entity on many occasions remains undetected till major complications supervene (Dutta, 2013; Mukhopadhyay *et al.*, 2014).

Chronic hypertension occurs more frequently with advancing age, and can be classified as hypertension secondary to other known causes or conditions or as primary (essential) hypertension with no known cause. Chronic hypertension is defined as hypertension that is present and observable before pregnancy or that is diagnosed before the 20th week of gestation. Primary hypertension accounts for 90% of chronic hypertension in pregnancy, while the remainder (10%) is secondary to underlying physiologic disorders such as renal disease (Dutta, 2013).

The National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy classified hypertensive disorders during pregnancy into 4 categories: preeclampsia/eclampsia, gestational hypertension, chronic hypertension and preeclampsia superimposed on chronic hypertension (Dutta, 2013; Mustafa *et al.*, 2012).

2.2 PREGNANCY-INDUCED HYPERTENSION: AN OVERVIEW

Pregnancy induced hypertension (PIH) is one of the leading causes of mortality and morbidity amongst pregnant women. Pregnancy-induced hypertension is a generic term used to define significant rise in blood pressure (systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg) during pregnancy, occurring after 20 weeks of gestation in a woman without prior hypertension. When accompanied by significant proteinuria, the disorder is termed preeclampsia and when it is without significant proteinuria, it is termed gestational hypertension. PIH occurs in about 5% to 8% of all pregnancies and more severe cases are frequently associated with poor fetal and maternal outcomes both in developed and developing countries. This renders PIH a cause for great concern to public health in general and maternal and child health nursing in particular (Dutta, 2013).

2.2.1 Gestational Hypertension

It is the most frequent of the hypertensive conditions of pregnancy with prevalence between 6 and 15% in nulliparous and 2-4% in multiparas. Gestational hypertension is the presence of hypertension in pregnancy without proteinuria, occurring after 20 weeks of gestation. The absence of proteinuria is a characteristic of this condition that differentiates it from preeclampsia, and the characteristic feature that differentiates gestational hypertension from chronic hypertension is the onset of the problem after 20 weeks and the absence of hypertension before pregnancy (Bansode, 2012).

A rigorous definition of severe gestational hypertension requires that the elevated blood pressure should be observed for at least 6 hours Gestational hypertension may be mild or severe. The condition is considered to be severe if there are sustained blood pressure elevations of systolic blood pressure to 160 mmHg or more and/or diastolic blood pressure to 110 mmHg or more.

(Dutta, 2013).

2.2.2 Preeclampsia

Although the exact pathophysiologic mechanism is not clearly understood, preeclampsia is primarily a disorder of placental dysfunction leading to a syndrome of endothelial dysfunction with associated vasospasm. Preeclampsia is a multisystem disease that is usually manifest as hypertension and proteinuria. It is peculiar to pregnancy, of placental origin and cured only by delivery. Blood vessel endothelial cell damage, in association with an exaggerated maternal inflammatory response, leads to vasospasm, increased capillary permeability and clotting dysfunction. These can affect all the maternal organs to varying degrees and account for all manifestations and complications (Bansode, 2012; Dutta, 2013; Uzan *et al.*, 2011).

The disease is progressive, but variable and unpredictable. Hypertension usually precedes proteinuria, a relatively late sign. Increased vascular resistance accounts for the hypertension, increased vascular permeability for proteinuria, reduced placental blood flow for intrauterine growth restriction (IUGR) and reduced cerebral perfusion for eclampsia. Some women develop this life-threatening disease at 24 weeks; others merely develop mild hypertension at term. Although only partly reflecting the severity of disease, the degree of hypertension can be used to help assess it (Dutta, 2013; Uzan *et al.*, 2011).

2.3 EPIDEMIOLOGY AND RISK FACTORS OF PIH

The prevalence of PIH varies with the population. A systematic review by the World Health Organization (WHO) indicates that hypertensive disorders of pregnancy account for 16% of all maternal deaths in developed countries, 9% of maternal deaths in Africa and Asia, and as many as 26% of maternal deaths in Latin America and the Caribbean (Jeyabalan, 2013). Hypertensive

disorders of pregnancy, including preeclampsia, consist of a broad spectrum of conditions that are associated with substantial maternal, fetal and neonatal morbidity and mortality. (Jeyabalan, 2013). In Ghana, the incidence of preeclampsia has been reported to be about 7.03% (Obed & Aniteye, 2006). Another study conducted in Ghana has also shown that the prevalence of gestational hypertension, preeclampsia and pregnancy induced hypertension were 5.87%, 6.55% and 12.42% respectively and with seasonal variations in their occurrence (Ahenkorah, 2009). Although most cases of preeclampsia occur in the absence of a family history, the presence of preeclampsia in a first-degree relative increases a woman's risk of severe preeclampsia two to four -fold. A history of preeclampsia in the father's mother also confers an increased risk. Most cases of preeclampsia occur in healthy nulliparous women, in whom the incidence of preeclampsia may be as high as 7.5%. Multiparous women pregnant with a new partner have a similar preeclampsia risk as nulliparous women; this has been ascribed to factors associated with a change in paternity or increased inter-pregnancy interval. In addition, women with preeclampsia in a prior pregnancy continue to have a high risk of preeclampsia in subsequent pregnancies (Wang *et al.*, 2009). Several medical conditions are associated with increased preeclampsia risk, including a medical history of chronic hypertension, kidney disease, diabetes, obesity, and hypercoagulable states, such as antiphospholipid syndrome and factor V Leiden.

Advanced maternal age (age ≥35 years), and pregnancy characteristics, such as twin or molar pregnancy, previous preeclampsia, or fetal congenital abnormality are also risk factors for PIH (Dutta, 2013; Wang *et al.*, 2009).

In the mother, preeclampsia may cause premature cardiovascular disease, such as chronic hypertension, ischemic heart disease, and stroke, later in life, while in children born after preeclamptic pregnancies, approximately 12–25% of fetal growth restriction and small-

forgestational-age infants as well as 15–20% of all preterm births are attributable to preeclampsia. Preeclampsia may be life-threatening for both mother and child, increasing both fetal and maternal morbidity and mortality. The associated complications of prematurity are substantial and include neonatal deaths and serious long-term neonatal morbidity. One-quarter of stillbirths and neonatal deaths in developing countries are associated with preeclampsia/eclampsia (Jeyabalan, 2013).

2.4 PATHOGENESIS AND PATHOPHYSIOLOGY OF PIH

2.4.1 Overview of the pathophysiology of PIH

Mechanisms of PIH are not clearly understood. An imposing number of mechanisms have been proposed to explain its cause. Those currently considered important include: placental implantation with abnormal trophoblastic invasion of uterine vessels; immunological maladaptive tolerance between maternal, paternal (placental), and fetal tissues; maternal maladaptation to cardiovascular or inflammatory changes of normal pregnancy; and genetic factors including inherited predisposing genes and epigenetic influences (Leveno, 2013; Wang *et al.*, 2009).

2.4.2 Abnormal placentation

The placenta is the central organ in the pathogenesis of preeclampsia. Preeclampsia only occurs in the presence of a placenta and almost always remits after its delivery. (Wang *et al.*, 2009). Pathological examination of placentas from women with severe preeclampsia showed evidence of placental hypo-perfusion and ischemia. Findings include acute atherosis, a lesion of diffuse vascular obstruction that includes fibrin deposition, intimal thickening, necrosis, atherosclerosis, and endothelial damage (Powe *et al.*, 2011). It is likely that some of the abnormalities seen in the preeclamptic placenta are consequences of the hypertension and endothelial injury induced by the disease. Placental infarcts, likely due to occlusion of spiral arteries, are also common. (Powe *et al.*, 2011; Wang *et al.*, 2009).

Early in normal placental development, extravillous cytotrophoblasts of fetal origin invade the uterine spiral arteries of the decidua and myometrium. Because of the necessity of the placenta in preeclampsia, there has been much scrutiny on how early abnormalities in placental vascular remodeling may play a role in the disease. These invasive cytotrophoblasts replace the endothelial layer of the maternal spiral arteries, transforming them from small, high-resistance vessels to high-caliber capacitance vessels capable of providing adequate placental perfusion to sustain the growing fetus. In normal placental development the cytotrophoblasts assume an endothelial phenotype in a process called pseudovasculogenesis, or vascular mimicry, by downregulating the expression of adhesion molecules characteristic of their epithelial cell origin and adopting an endothelial cell surface adhesion phenotype. In preeclampsia, this transformation is incomplete. Cytotrophoblast invasion of the spiral arteries is limited to the superficial decidua, and the myometrial segments remain narrow. In preeclampsia, cytotrophoblasts do not undergo this switching of cell-surface molecules and thus are unable to adequately invade the myometrial spiral arteries. This shallow invasion has been shown to be related to a failure of the cytotrophoblasts to adopt an endothelial adhesion phenotype (Powe *et al.*, 2011; Wang *et al.*, 2009).

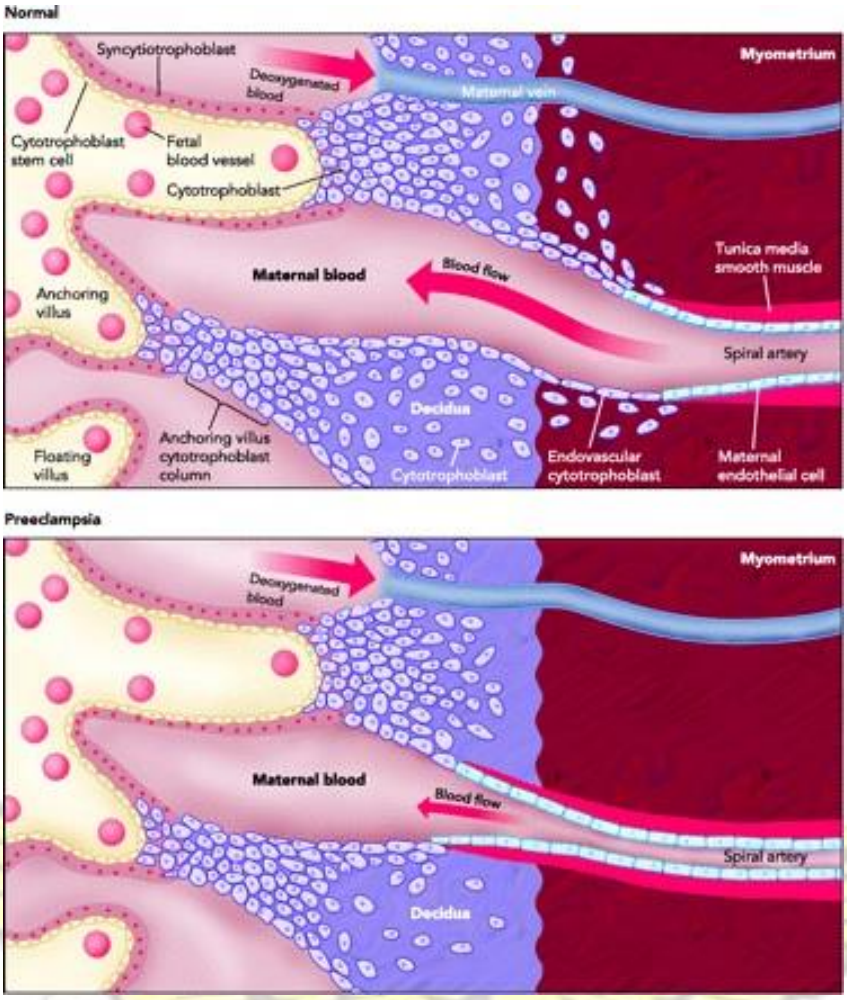


Figure 2.1 **Abnormal placentation in preeclampsia**

Source: (Wang *et al.*, 2009)

2.4.3 Endothelial Cell Injury

Many serum markers of endothelial activation and endothelial dysfunction are deranged in women with preeclampsia, including, cellular fibronectin, soluble tissue factor, soluble Eselectin, platelet-derived growth factor, and endothelin. Although preeclampsia appears to begin in the placenta, the target place is the maternal endothelium. (Wang *et al.*, 2009). Endothelium that is intact has anticoagulant properties, and endothelial cells blunt the response of vascular smooth muscle to agonists by releasing nitric oxide. Damaged or activated endothelial cells tend to produce less nitric

oxide and secrete substances that promote coagulation and increase sensitivity to vasopressors (Gant *et al.*, 1974).

Recently, it is recognized that women with preeclampsia demonstrate a vascular sensitivity to angiotensin type II, and a disrupted balance between vasodilation (prostacyclin) and vasoconstriction (thromboxane). The substances produced as a result of placental ischemia cause generalized endothelial dysfunction, as demonstrated by the following laboratory findings: elevated fibronectin levels, increased Factor VIII and thrombomodulin; reduction in vasodilatory and vasorelaxing substances; decreased nitrous oxide (NO) and prostacyclin (vasodilatory effect); increased production of endothelin and thromboxane (vasoconstrictive effect); and increased vascular response to angiotensin type II. (Dudenhausen, 2014). Further evidence of endothelial activation includes the characteristic changes in glomerular capillary, endothelial morphology, increased capillary permeability, and elevated blood concentrations of substances associated with endothelial activation. These latter substances are transferable, and serum from women with preeclampsia stimulates some of these substances in greater amounts. It seems likely that multiple factors in the plasma of preeclamptic women combine to have these vasoactive effects (Myers *et al.*, 2007; Walsh, 2009).

2.4.3.1 Nitric Oxide and Endothelin

Endothelin also acts as a potent peripheral vasoconstrictor, and endothelin-1 (ET-1) is the primary isoform produced by human endothelium. Endothelial dysfunction causes decreased expression of NO and increased expression ET-1, whereby ET-1 inactivates NO and contributes to vasoconstriction. Nitric Oxide (NO) is a potent vasodilator and platelet aggregation inhibitor that is synthesized from L-arginine by endothelial cells. Central phenomena are: generalized vasoconstriction, endothelial lesions with increased peripheral vascular resistance,

microcirculation disorders; activation of intravascular coagulation by fibrin deposits and platelet aggregation; hypoperfusion of the terminal vascular bed and tissue hypoxia in organs (Dudenhausen, 2014).

2.4.3.2 Prostaglandins

Several prostanoids are thought to be central to PIH. PIH is characterized by a marked displacement in favor of thromboxane A_2, and thus a predominating vasoconstriction pathophysiology. Compared with normal pregnancy, the production of endothelial prostacyclin, which have the physiological effects of potent vasodilation and inhibition of platelet aggregation, is decreased in preeclampsia. This action appears to be mediated by phospholipase A_2. The decrease in prostacyclin plays a role in hypertension, because peripheral vasodilation does not occur (Davidge *et al.*, 2014; Dudenhausen, 2014). In contrast, thromboxane A_2, which is produced in the placenta and causes vasoconstriction and platelet aggregation, is seven times higher in PIH than in normotensive pregnant women. (Dudenhausen, 2014). These changes are apparent as early as 22 weeks in women who later develop preeclampsia (Chavarria *et al.*, 2003).

2.4.4 Pro-angiogenic Factors and Vascular Homeostasis

Recently, circulating antiangiogenic proteins have been implicated in the pathogenesis of many of the maternal features of the disease. Abnormalities in the placenta and resulting consequences to the fetus are a hallmark of preeclampsia, but the maternal features of the disease have been its most mysterious feature. Angiogenic factors are thought to be important in the regulation of placental vascular development (Powe *et al.*, 2011). Trophoblast of women who are likely to develop preeclampsia overproduces at least two antiangiogenic peptides that enter the maternal circulation: soluble Fms-like tyrosine kinase 1 (sFlt-1) and soluble endoglin (sEng) (Karumanchi *et al.*, 2014).

The physiological role of PlGF is less well understood than that of VEGF, but PlGF appears to stimulate angiogenesis under conditions of ischemia, inflammation, and wound healing and may contribute to atherosclerosis. PlGF, with structural homology to VEGF-A, is a potent angiogenic growth factor that is thought to amplify VEGF signaling by displacing VEGF from the Flt1 receptor and allowing it to bind to the more potent KDR (VEGFR-2, murine Flk-1) receptor instead. Whereas VEGF binds to both Flt-1 and KDR receptors, PlGF homodimers bind exclusively to Flt-1. Soluble Fms-like tyrosine kinase 1 (sFlt-1) is a variant of the Flt-1 receptor for placental growth factor (PlGF) and for vascular endothelial growth factor (VEGF). Flt1 (VEGFR-1) and VEGFR-2 are essential for normal placental vascular development (Karumanchi *et al.*, 2014). Alterations in these pathways in early gestation may contribute to inadequate cytotrophoblast invasion observed in the placentas of women with preeclampsia. VEGF stabilizes endothelial cells in mature blood vessels and is particularly important in maintaining the endothelium in the kidney, liver, and brain. VEGF signals through two major receptors: Flk and Flt1. (Powe *et al.*, 2011; Wang *et al.*, 2009). Increased maternal sFlt-1 levels inactivate and decrease circulating free PlGF and VEGF concentrations leading to endothelial dysfunction (Maynard *et al.*, 2003). Invasive cytotrophoblasts express vascular endothelial growth factor (VEGF), placental growth factor (PlGF), and VEGFR-1 (Flt-1); expression of these proteins by immunolocalization is altered in preeclampsia. sFlt1 has been shown to decrease cytotrophoblast invasiveness *in vitro*, and circulating sFlt1 levels stay relatively low early in pregnancy and begin to rise in the third trimester. This may reflect a physiological anti-angiogenic shift in the placental milieu toward the end of pregnancy, corresponding to the completion of the vasculogenic phase of placental growth. Alterations in these angiogenic pathways in early gestation could contribute to the inadequate cytotrophoblast invasion seen in preeclampsia, thereby sparking a cycle of

continued derangement in angiogenic balance; however, there is no definitive evidence for this hypothesis so far. By the third trimester, excess placental sFlt1 accumulates in the maternal circulation, produces end-organ effects, and reflects the degree of placental ischemia (Karumanchi *et al.*, 2014; Powe *et al.*, 2011; Wang *et al.*, 2009).

In preeclampsia, excessive production of surface endoglin leads to increased sEng in the maternal circulation. sEng together with sFlt1 may be responsible for the maternal endothelial dysfunction and the clinical manifestations of preeclampsia Soluble endoglin (sEng) is a placenta-derived 65-kDa molecule that blocks endoglin (CD105), which is a surface co-receptor for the transforming growth factor-β (TGFβ) family. This soluble form of endoglin inhibits various TGFβ isoforms (TGFβ1, TGFβ3) from binding to endothelial receptors and results in decreased endothelial nitric oxide-dependent vasodilatation. (Powe *et al.*, 2011; Venkatesha *et al.*, 2006). Serum levels of sEng also begin to increase months before clinical preeclampsia develops (Haggerty *et al.*, 2012)

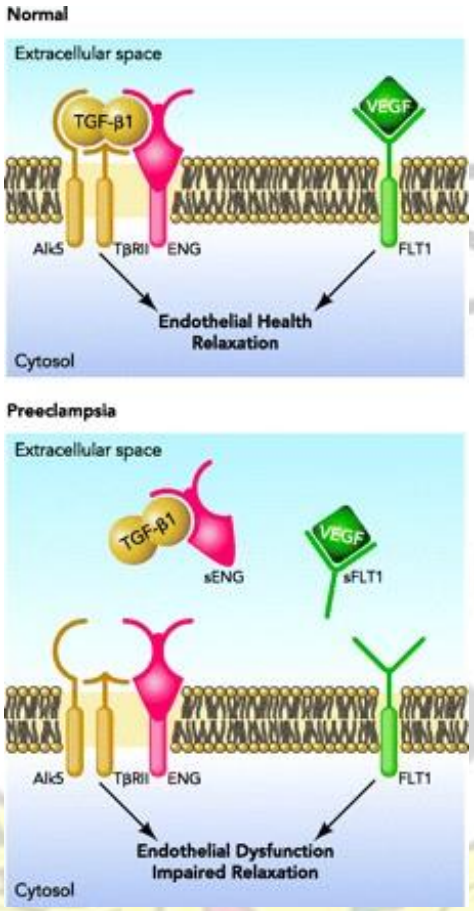


Figure 2.2: **sFlt1 and sEng cause endothelial dysfunction by antagonizing VEGF and TGFβ signaling**

Source: (Wang *et al.*, 2009).

2.4.5 Immunological Factors

Normal placentation requires the development of immune tolerance between the fetus and the mother. Immune maladaption remains an intriguing explanation about the pathogenesis of PIH. (Wang *et al.*, 2009). The incidence of PIH in nulliparae is considerably higher than in parous women with the same partner, but it increases markedly with a partner change. The confrontation of the mother with paternal or fetal antigens appears to play a protective role. The finding that the duration of sexual intercourse, and thus exposure to the father's sperm, is inversely proportional to the risk of preeclampsia, must be similarly interpreted (Dudenhausen, 2014). In addition, women

using barrier contraceptive methods that reduce maternal exposure to sperm have increased incidence of preeclampsia (Wang *et al.,* 2009). Women with untreated HIV have a very low incidence of preeclampsia, but the incidence returns to normal in HIV-positive women who are on antiretroviral therapy (Powe *et al.*, 2011; Wang *et al.*, 2009). Natural killer (NK) cells at the maternal/fetal interface are also thought to play an important role in the pathogenesis of preeclampsia. They are thought to promote angiogenesis and are involved in trophoblast invasion, which may contribute to the abnormal placental development seen in the disease. Recent genetic studies have suggested that the susceptibility to preeclampsia may be influenced by polymorphic human leukocyte antigen C (HLA-C) ligands and the killer immunoglobulin receptors (KIRs) present on NK cells. Alterations in decidual NK-cell signaling with subsequent disturbance in the secretion of cytokine and angiogenic factors may mediate the abnormal placentation noted in preeclampsia. Normal placentation requires an immune tolerance for fetal antigen, which may be altered in preeclampsia, because pathological examination of preeclamptic placentas reveals increased dendritic cell and macrophage infiltration as well as signs of chronic inflammation. (Dudenhausen, 2014; Leveno, 2013; Powe *et al.*, 2011; Wang *et al.*, 2009).

2.4.6 Genetic Factors

The hereditary predisposition for preeclampsia likely is the result of interactions of literally hundreds of inherited genes - both maternal and paternal - that control myriad enzymatic and metabolic functions throughout every organ system. From a hereditary viewpoint, preeclampsia is a multifactorial, polygenic disorder. Plasma-derived factors may induce some of these genes in preeclampsia (Mackenzie *et al.*, 2012)

Although most cases of preeclampsia occur in women without a family history, primigravidae whose mothers and sisters suffered from preeclampsia have a 2 to 4-fold increased risk of suffering

from preeclampsia themselves According to a number of observations, a genetic predisposition seems a likely cause of PIH. (Wang *et al.*, 2009). These studies showed that both maternal and paternal influences on fetal genes probably contribute to inadequate trophoblast invasion and subsequent preeclampsia. If a woman is impregnated by a man whose earlier partner had preeclampsia, her risk of developing preeclampsia is almost doubled. Also, partners of men who are the products of a pregnancy complicated by preeclampsia have an increased preeclampsia risk. (Dudenhausen, 2014; Wang *et al.*, 2009). While the evidence is not conclusive, some genes have been implicated in the pathogenesis of PIH: T 235 variant of the angiotensinogen gene, the endothelial nitric oxide synthase (NOS) gene, and genes which cause thrombophilia (Dudenhausen, 2014).

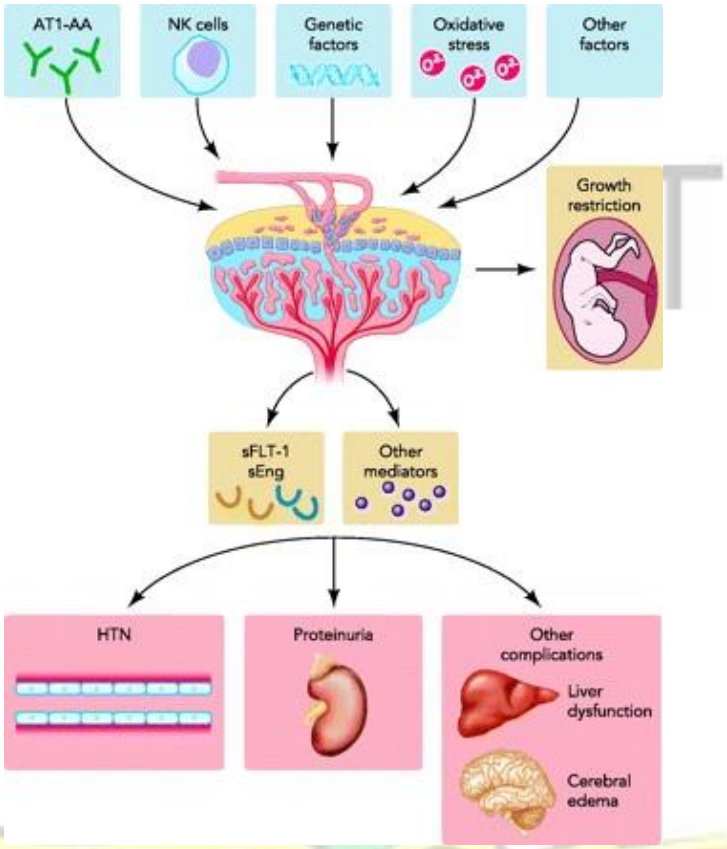


Figure 2.3: Summary of the pathogenesis of preeclampsia

Source: (Wang *et al.*, 2009)

2.5. ADVERSE EFFECTS OF PIH

2.5.1 Overview of Intrauterine Growth Restriction (IUGR)

Growth (an increase in the number and size of cells or in the mass of tissues) and development (changes in the structure and function of cells or tissues) of the fetus are complex biological events influenced by genetic, epigenetic, maternal maturity, as well as environmental and other factors. These factors affect the size and functional capacity of the placenta, uteroplacental transfer of nutrients and oxygen from mother to fetus, conceptus nutrient availability, the fetal endocrine milieu, and metabolic pathways (Wu *et al.*, 2006).

Intrauterine growth restriction (IUGR) can be defined as impaired growth and development of the mammalian embryo/fetus or its organs during pregnancy. IUGR indicates that there is a pathological process operating to restrict the growth rate of the fetus (Wu *et al.*, 2006).

In the past, infants who were small for gestational age were designated as suffering from intrauterine growth retardation. To avoid undue alarm in parents to whom the term "retardation" implies abnormal mental function, this term has been replaced by "fetal growth restriction (FGR)," or intrauterine growth restriction (IUGR) Paediatricians use the term "small for gestational age" (SGA) to define newborns with a birth weight less than the 10th percentile for their gestational age (Dudenhausen, 2014; Leveno, 2013).

Clinically, a differentiation is made between symmetrically and asymmetrically growth-restricted newborns: in symmetrically grown- restricted babies, all body measurements are equally growth restricted, and it is assumed that the growth restriction began around mid-pregnancy; in the asymmetrically grown- restricted babies, length is appropriate for gestational age, whereas weight is low. This growth restriction occurs in the last weeks of the pregnancy (Leveno, 2013).

2.5.2 Incidence of Intrauterine Growth Restriction: An Overview

The incidence of IUGR varies according to the population under examination, the geographic location, the standard growth curves used as reference, and the percentile chosen to indicate abnormal growth (i.e., the 3rd, 5th, 10th, or 15th) (Kramer, 1987). The incidence of IUGR should be, by definition, close to 10% of all births. However, only 20–30% of these fetuses are small because of a pathological restriction of their growth (Dudenhausen, 2014). It has been reported that approximately one-fourth to one-third of all infants weighing less than 2500 g at birth have sustained IUGR, and approximately 4% to 8% of all infants born in developed countries and 6%

to 30% of those born in developing countries have been classified as growth restricted (Kramer, 1987).

2.5.3 Etiology of Intrauterine Growth Restriction

IUGR encompasses many different maternal and fetal entities. Some can be detected before birth, whereas others can be found only at autopsy. It is important to discern the cause of IUGR, because in many cases subsequent pregnancies may also be affected. The most common causes of IUGR are placental vascular insufficiency, fetal genetic conditions, and maternal conditions (Arias *et al.*, 2008; Baker & Kenny, 2011).

2.5.3.1 Placental Abnormalities

Abnormalities of the placenta, affecting the maternal or the fetal circulation or both are among the most common causes (75%–80% of the cases) of IUGR. In the majority of these cases, there is diminished maternal uteroplacental blood flow caused by insufficient or incomplete trophoblastic invasion of the spiral arteries in the placental bed. This leads to chronic placental insufficiency with inadequate substrate transfer. Trophoblastic cells, under normal conditions first infiltrate the decidua and then the myometrial portion of the spiral arteries, destroying the elastic and muscular layers and replacing them with fibrinoid material. Another feature of abnormal placentation is the deposition of lipoprotein and the infiltration by foamy macrophages of the vascular wall, giving the appearance of accelerated atherosclerosis. The rigid vessel walls are transformed into flaccid sac-like structures that can accommodate the increased uteroplacental blood flow that occurs during pregnancy. These transformed spiral arteries are not affected by maternal vaso-regulatory mechanisms. The initial phase of the trophoblastic invasion of the spiral arteries usually ends by the 16th week of gestation, but in many cases completion of the adaptative changes does not occur until 20–22 weeks. When placentation is abnormal, trophoblastic invasion is largely confined to

the decidual layer with absent or incomplete changes in the myometrial portion of the spiral and radial arteries. The presence of spiral and radial arteries with intact muscular and elastic layers causes increased vascular resistance and decreased blood flow to the intervillous space, restricting the maternal capacity to provide oxygen and nutrients to the fetus. Also, the vessels with absent or incomplete transformation remain reactive to vasoactive substances produced or ingested by the mother (Arias *et al.*, 2008; Dudenhausen, 2014).

2.5.3.2 Maternal Conditions

The maternal conditions associated with IUGR interfere with fetal growth by one of the three mechanisms: causing or aggravating placental vascular insufficiency; limiting the availability of substrates required for fetal growth and development; or transferring to the fetus substances that affect the fetal growth (Arias *et al.*, 2008; Baker & Kenny, 2011).

2.5.3.2.1 Maternal diseases

The mechanism explaining the development of placental vascular lesions in maternal conditions remains unknown. Maternal conditions such as preeclampsia, chronic hypertension, chronic renal disease, connective tissue disorder, diabetes with vascular lesions, sickle cell anemia and cardiac disease class III or IV are associated with placental vascular insufficiency. The common link between these conditions and placental vascular insufficiency may be an underlying alteration of the maternal or the fetal hemostatic systems or vasoconstriction, causing ischemic infarcts and decreased perfusion. Irrespective of the mechanism, the practical point is that the association between maternal chronic medical conditions, placental vascular insufficiency, and IUGR is robust and surveillance of fetal growth is a fundamental aspect of the prenatal care of women afflicted with these conditions (Arias *et al.*, 2008; Karumanchi *et al.*, 2014).

2.5.3.2.2 Maternal nutrition before and during pregnancy

Critical substrate requirement for fetal growth such as glucose, amino acids and oxygen are deficient during pregnancy. This is an important cause of IUGR in women with under-nutrition. There are some maternal conditions that cause IUGR by limiting the availability of substrates which the fetus requires for normal growth and development. One of these conditions is severe maternal malnutrition. The importance of maternal nutrition in fetal growth and birth weight was demonstrated by studies in Russia and Holland, where women suffered inadequate nutrition during World War II. Studies on the siege of Leningrad during World War II and the Dutch famine during the winter of 1944 demonstrated that severe protein-caloric malnutrition, especially during the second half of the pregnancy, causes decreased fetal weight (Arias *et al.*, 2008; Karumanchi *et al.*, 2014).

2.5.3.2.3 Environmental Toxins

Maternal alcohol ingestion is another well-recognized cause of IUGR due to the effects of a toxin upon the fetus. Reduction in birth weight also occurs with maternal alcohol ingestion of as little as one to two drinks per day. The alcohol effect is synergistic with that from smoking. In one series of 76 babies with fetal alcohol syndrome, IUGR occurred in 91%. The fetal effects of alcohol are more severe in heavy drinkers. The mechanism behind the decrease in fetal growth caused by maternal cigarette smoking has not been clarified, but it probably results from a combination of factors such as reduced intervillous blood flow, the effect of carbon monoxide and thiocyanate on the fetus, and reduced prostacyclin production. A third group of maternal conditions affect fetal growth by supplying the fetus with substances toxic for growth and development. A classical example of this group is maternal cigarette smoking. For women who smoke during pregnancy, the reduction in fetal growth at term reaches between 150 and 400 g, but the effect of smoking is evident at every gestational age. If smoking is stopped before the third trimester, its adverse effect

on birth weight is reduced. Tobacco-chewing gravidas and women exposed to second-hand smoking also have reduced fetal weigh (Arias *et al.*, 2008; Baker & Kenny, 2011).

The use of certain medications during pregnancy is also associated with IUGR. Cancer chemotherapeutic agents, Coumadin, and phenytoin (Dilantin) are drugs that have the potential to cause IUGR The chronic ingestion of heroin, morphine, cocaine, and other addictive substances is also frequently associated with FGR. Similar to alcohol and tobacco the mechanism most probably involves a direct drug effect on the fetus in addition to maternal malnutrition which is common in drug abusers. (Arias *et al.*, 2008).

2.5.3.3 Fetal Abnormalities

Fetal abnormalities arise when there is enough substrate in the maternal blood and also cross the placenta but are not utilized by the fetus. The failure of non-utilization may be due to: structural anomalies either cardiovascular, renal or others; chromosomal abnormality is associated with 8 - 12% of growth retarded infants, and the common abnormalities are triploidy and aneuploidy (Trisomies [13, 18, 21] and Turner's syndrome are commonly observed); infection; and multiple pregnancy (there is mechanical hindrance to growth and excessive fetal demand) (Arias *et al.*, 2008; Dutta, 2013).

2.5.3.3.1 Chromosomal abnormality

The possibility of a fetal congenital disorder should always be considered in patients with "idiopathic" or "unexplained" IUGR. IUGR is common in chromosomal disorders, especially in somatic trisomies. It also occurs in patients with familial dysautonomy, osteogenesis imperfecta, and other multifactorial disorders. Single gene mutations do not affect fetal growth as much as do chromosomal defects. The most common abnormalities associated with IUGR were chromosomal, particularly trisomy 18. The mechanism of fetal growth impairment secondary to genetic

syndromes is unknown but it is possible that chromosomal defects cause alterations in placental function, resulting in fetal malnutrition (Arias *et al.*, 2008).

2.5.3.3.2 Fetal infections

The viral infections associated with impaired fetal growth are congenital rubella, cytomegalic virus, congenital varicella, human immunodeficiency virus, and acute herpes simplex virus infections. Fetal infections are not a common cause of IUGR. Bacterial infections usually have acute courses and cause preterm labor, preterm rupture of membranes, or fetal death. Viral infections on the other hand may be chronic and affect fetal growth. Protozoan infections resulting from Toxoplasma gondii, Plasmodium sp., or Trypanosoma cruzi (Chagas disease) reportedly can cause IUGR (Arias *et al.*, 2008; Dutta, 2013).

2.5.3.3.3 Multiple Gestation

There is mechanical hindrance to growth and excessive fetal demand. It has long been recognized that multiple pregnancies are associated with a high progressive decrease in fetal and placental weight as the number of offspring increases in humans. The decrease in weight of twin fetuses, frequently with mild IUGR, is usually due to decreased cell size; the exception is severe IUGR associated with monozygosity and vascular anastomoses, wherein cell number also may be decreased. Twins with mild IUGR have an acceleration of growth after birth, so that their weight equals the median weight of singletons by 1 year of age These changes in twins are similar to those seen in IUGR secondary to poor uterine perfusion or maternal malnutrition.

(Arias *et al.*, 2008; Baker & Kenny, 2011).

Table 2.1: Smoothed Percentiles of Estimated Birth Weight (grams) for Gestational Age

	Percentile				
Age (wk)	5th	10th	50th	90th	95th
20	249	275	412	772	912
21	280	314	433	790	957
22	330	376	496	826	1023
23	385	440	582	882	1107
24	435	498	674	977	1223
25	480	558	779	1138	1397
26	529	625	899	1362	1640
27	591	702	1035	1635	1927
28	670	798	1196	1977	2237
29	772	925	1394	2361	2553
30	910	1085	1637	2710	2847
31	1088	1278	1918	2986	3108
32	1294	1495	2203	3200	3338
33	1513	1725	2458	3370	3536
34	1735	1950	2667	3502	3697
35	1950	2159	2831	3596	3812
36	2156	2354	2974	3668	3888
37	2357	2541	3117	3755	3956
38	2543	2714	3263	3867	4027
39	2685	2852	3400	3980	4107
40	2761	2929	3495	4060	4185
41	2777	2948	3527	4094	4217
42	2764	2935	3522	4098	4213
43	2741	2907	3505	4096	4178
44	2724	2885	3491	4096	4122

Source: (Arias *et al.*, 2008; Cunningham *et al.*, 2014; Leveno, 2013).

2.6 LOW BIRTH WEIGHT (LBW)

2.6.1 Overview of LBW

Birth weight is governed by two major processes: duration of gestation and intrauterine growth rate. LBW is thus caused by either a short gestation period or intrauterine growth restriction (or a combination of both). Low birth weight, defined as weighing less than 2,500 g at birth, remains a significant public health problem in many parts of the world and is associated with a range of both short and long-term adverse consequences. Prematurity is usually defined as a gestational age of less than 37 weeks. Although about one-half of all LBW infants in industrialized countries are born preterm (<37 weeks gestation), most LBW infants in developing countries are born at term and are affected by intrauterine growth restriction that may begin early in pregnancy. The birth weight and gestational age each act as a powerful predictor of infant growth and survival, and is dependent on maternal health and nutrition during pregnancy. The more severe the growth restriction within the LBW category, the higher the risk of death. LBW is a strong predictor for size in later life because IUGR infants seldom catch-up to normal size during childhood. Other important causes could include maternal infections, low maternal nutrient intake, higher nutrient losses, and/or increased nutritional requirements during pregnancy. Maternal nutrition is an important factor from a public health point of view because it is modifiable and therefore susceptible to public health interventions (Bergner & Susser, 1970; Kramer, 1987; Ramakrishnan, 2004).

2.6.2 Maternal anthropometry and pregnancy outcome

Pre-pregnancy weight, body mass index (BMI) and gestational weight gain all have strong, positive effects on foetal growth suggesting that energy balance is an important determinant of birth outcomes. The causes of low birth weight are complex and interdependent, but the anthropometry of the mother and her nutritional intake are thought to be among the most important. (Lammi-Keefe *et al.*, 2008; Ramakrishnan, 2004).

2.6.3 Macronutrient supplementation before and during pregnancy

Evidence from systematic reviews of randomized controlled trials on the effectiveness of nutritional interventions aimed at reducing IUGR has demonstrated the beneficial effects of macronutrient (protein/energy) supplementation, with an overall odds ratio of 0.77 (95% CI 0.58, 1.01) for reducing IUGR. Supplementation was associated with increases in maternal weight gain and mean birth weight, and a decrease in the number of LBW babies of borderline significance. Poor nutrition is a known cause of LBW, especially in developing countries. An adequate nutrient supply to the foetus is essential in enhancing birth weight. There is controversy on whether dietary macronutrient and micronutrient supplementation in pregnancy can increase birth weight (Lammi-Keefe *et al.*, 2008). A meta-analysis showed only modest increases in maternal weight gain and foetal growth following dietary supplementation. (Lammi-Keefe *et al.*, 2008).

2.6.4 Micronutrient supplementation before and during pregnancy: vitamin B_{12} and folate

Several randomized trials have demonstrated the effect of micronutrients in significantly decreasing the risk of low birth weight and the need for multiple micronutrient supplementations of pregnant women (Lammi-Keefe *et al.*, 2008). Some studies have recently observed that specific micronutrients are possible limiting factors for foetal growth. (Muthayya, 2009).

The form of vitamin B_{12} most frequently used in supplements and/or fortified foods is cyanocobalamin, which is readily converted in the body to its utilizable forms of methylcobalamin and 5-deoxyadenosylcobalamim. Other supplemental forms include methylcobalamin and adenosylcobalamin. Vitamin B_{12} is synthesized by bacteria and found primarily in meat, eggs, fish (including shellfish), and to a lesser extent dairy products. Fortified breakfast cereals provide a significant source of vitamin B_{12} (6.0 mcg/3/4 cup), particularly for vegetarians. Plant sources, such as spirulina (algae) and nori (seaweed), contain vitamin B_{12} analogues, which can compete

with vitamin B_{12} and inhibit metabolism. Although vitamin B_{12} deficiency is not a common finding among women of childbearing age, women who avoid animal-based foods, the sole source of vitamin B_{12}, should be advised to take supplemental vitamin B_{12} (Lammi-Keefe *et al.*, 2008).

Folate is a water-soluble vitamin occurring either naturally in food or as folic acid, which is the synthetic form in supplements or fortified foods. Folate must be consumed in adequate amounts prior to and during pregnancy to ensure an optimal pregnancy outcome (Lammi-Keefe *et al.*, 2008; Tamura & Picciano, 2006). In addition to the role of folic acid in the development of the neural tube, which takes place during the first 28 days of gestation, folate is of vital importance throughout gestation for a positive pregnancy outcome. DNA synthesis is dependent on folate and when intake is limited, cell division slows down at a time when the developing embryo has the greatest need (Lammi-Keefe *et al.*, 2008).

Folate is required for the formation of red blood cells, and the expansion in the number of red blood cells for maternal and fetal circulation further increases the requirement for folate during pregnancy.Folate requirements are increased in pregnancy to meet the demands for increased DNA synthesis and thus cell division (Scholl & Johnson, 2000). The increase in cell division is associated with the rapidly growing fetus and placenta coupled with the increasing size of the maternal reproductive organs. Restricted folate intake during pregnancy has been associated with poor pregnancy outcomes including preterm delivery, low infant birth weight, and fetal growth retardation (Lammi-Keefe *et al.*, 2008; Scholl & Johnson, 2000). Naturally occurring dietary folate is concentrated in certain foods, including orange juice, dark green leafy vegetables, and dried beans such as black beans and kidney beans. With the exception of liver, meat is generally not a good source of folate. Pregnant women consume folate as naturally occurring food folate, folic

acid in fortified foods, and supplements that contain folic acid. (Lammi-Keefe *et al.*, 2008; Suitor & Bailey, 2000).

2.6.5 Role of Other factors

The role of infections in particular is interesting; for example, malaria prophylaxis may reduce LBW in primigravidae. The etiology of LBW is complex and may vary by setting. Several nonnutritional factors, such as infections, hypertension, smoking, and environmental factors (such as indoor air pollution due to cooking smoke and poor housing quality) are known determinants. (Bergner & Susser, 1970; Ramakrishnan, 2004).

2.7 HOMOCYSTEINE

2.7.1 Definition and structure

Homocysteine is a homologue of the amino acid cysteine, differing by an additional methylene (CH2-) group. Homocysteine is a sulphur-containing amino acid that is formed after the demethylation of methionine. It has the formula HOOC-CH(NH_2)-CH_2-CH_2-S-H (Selhub & Mayer, 1999).

2.7.2 Biosynthesis of homocysteine (Hcy)

Methionine is an essential amino acid that is found in the highest quantities in animal products such as eggs, meat, fish, and milk at levels around 3 g/100g of protein. Methionine is also found in most plant sources such as fruit, vegetables, nuts, and cereals at levels around 1 g/100 g of protein. Consequently, a meal very high in protein and methionine (over 50 grams) can elevate plasma homocysteine levels. Methionine is the only known precursor of Hcy in human diet. Homocysteine is not obtained from the diet. Instead, it is biosynthesized from methionine in a 3step reaction, which includes activation of methionine by ATP, loss of methyl group and enzymatic hydrolysis (Selhub & Mayer, 1999).

First, methionine receives an adenosine group from ATP, a reaction catalyzed by S-adenosylmethionine synthase, to give S-adenosyl methionine (SAM). SAM is the novel methyl donor in many biological reactions. SAM then transfers the methyl group to an acceptor molecule, (i.e., norepinephrine as an acceptor during epinephrine synthesis, DNA methyl-transferase as an intermediate acceptor in the process of DNA methylation) and is converted into S-adenosylhomocysteine (SAH), which can be hydrolysed into Hcy. The trans-methylation pathway is the sole metabolic pathway that is known to produce Hcy in the body (Selhub & Mayer, 1999).

2.8 METABOLISM OF HOMOCYSTEINE

The metabolism of homocysteine is at the intersection of two metabolic pathways: remethylation and trans-sulphuration. In remethylation, homocysteine acquires a methyl group from betaine or methyltetrahydrofolate to form methionine, and remethylation of homocysteine to methionine is catalyzed by betaine-homocysteine methyl-transferase (BHMT) in the liver or by methionine synthase (MS) in most body tissues, with the later depending on methyltetrahydrofolate as a methyl donor and vitamin B_{12} as a co-factor. The enzyme methelenetetrahydrofolate reductase (MTHFR) catalyzes the synthesis of methyltetrahydrofolate, in the presence of vitamin B_{12}. It appears that all tissue types have the ability to re-methylate homocysteine using this pathway (Ciaccio *et al.*, 2008; Finkelstein, 1998; Selhub & Mayer, 1999).

The trans-sulfuration pathway uses the enzyme cystathionine synthase (CBS) and vitamin B_6 as a co-factor to irreversibly convert homocysteine into cystathionine. In a state of methionine excess, homocysteine condenses with serine to form cystathionine in an irreversible reaction catalyzed by cystathionine beta synthase (CBS) and vitamin B_6. Cystathionine is converted into the amino acid cysteine. Cysteine is used in a variety of metabolic pathways or converted into inorganic sulfate and excreted in urine (Ciaccio *et al.*, 2008; Selhub & Mayer, 1999).

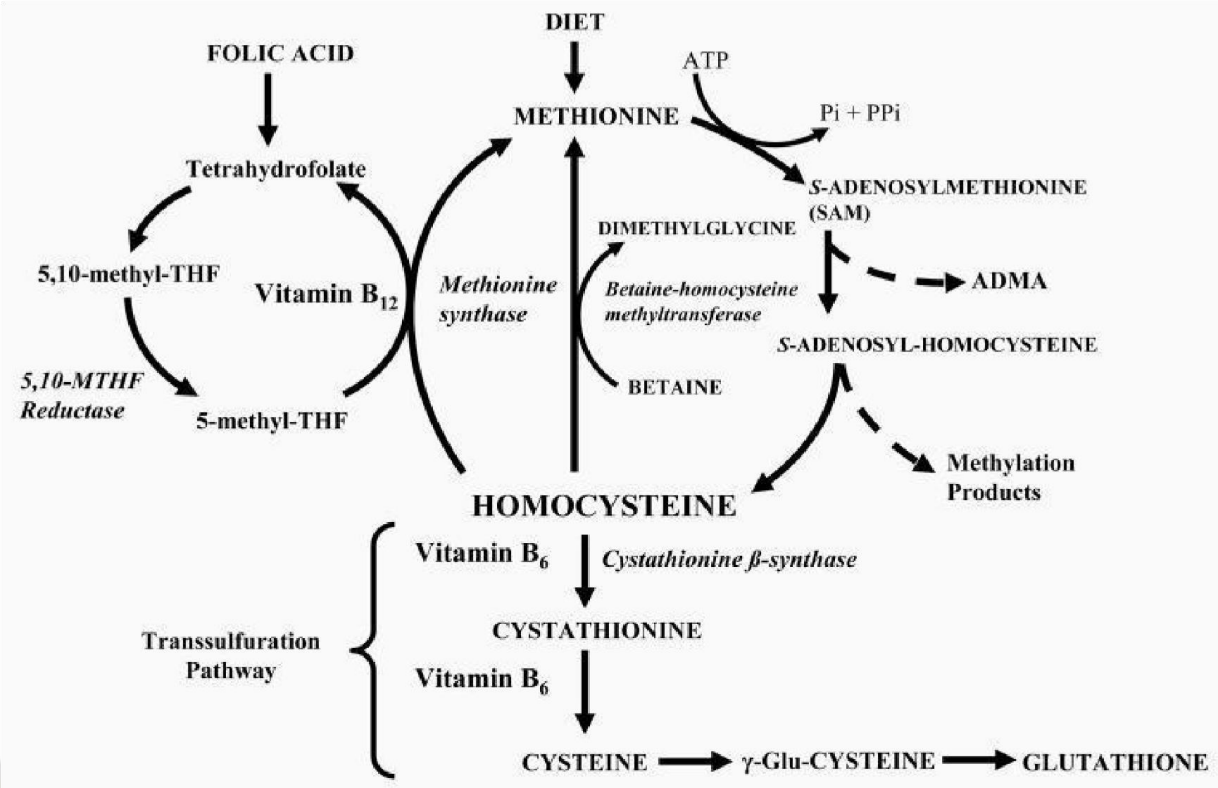


Figure 2.4: Mechanism of homocysteine production and metabolism Source:

(Maron & Loscalzo, 2009).

2.9 Hyperhomocysteinemia

2.9.1 Overview of Hyperhomocysteinemia

Numerous clinical and epidemiological studies have indicated that hyperhomocysteinaemia is an independent risk factor for cardiovascular disease. Hyperhomocysteinemia is a pathological condition characterized by an increase in plasma concentration of total homocysteine (15 mol/L). (Shai *et al.*, 2004). Deficiencies of the vitamins folic acid (B_9), pyridoxine (B_6), or B_6 (cyanocobalamin) can lead to hyperhomocysteinaemia**.** Hyperhomocysteinemia also occur in the rare hereditary disease homocysteinuria and in the methylenetetrahydrofolate reductase polymorphism genetic traits (Austin *et al.*, 2004; Lawrence-de-Koning *et al.*, 2003)

2.9.2 The Pathogenesis of Hyperhomocysteinaemia

Elevated homocysteine levels are caused by four major factors, including genetic deficiencies in enzymes involved in homocysteine metabolisms such as cystathionine β synthase (CBS), methionine synthase (MS), and methelenetetrahydrofolate reductase (MTHFR); nutritional deficiency in vitamins (B_6, B_{12} and folate); renal failure for effective amino acid clearance; drug interactions such as nitric oxide, methotrexate and phenytoin that interfere with Hcy metabolisms (Lawrence-de-Koning *et al.*, 2003)

2.9.2.1 Genetic deficiencies

The metabolism of homocysteine is dependent on one of several enzymes, a methyl donor, and several nutrient cofactors. All of these pathways are therefore ultimately controlled by the genes encoding the various metabolic enzymes, and, as with any gene, there are inborn errors that affect the efficiency by which homocysteine can be metabolized. Of all the determinants, genetic mutations have the largest capacity to affect plasma homocysteine levels. A defect at any point in homocysteine metabolism can lead to hyperhomocysteinaemia or homocysteinuria. Three main errors that have become clinically important are cystathionine β-synthase deficiency, inborn errors of cobalamin metabolism or absorption, and inborn errors in folate metabolism Cystathionine β-synthase (CBS) deficiency is the most common genetic cause of hyperhomocysteinaemia (Lawrence-de-Koning *et al.*, 2003; Selhub & Mayer, 1999). Homozygous CBS deficiency is inherited as an autosomal recessive disorder. While homozygous CBS deficiency is rare, heterozygous CBS deficiency occurs in approximately 1% of the general population and is associated with premature atherosclerosis and thrombotic disease in phenotypically normal individuals. Deficiency of 5,10-methylenetetrahydrofolate reductase (MTHFR), the enzyme

involved in folate-dependent remethylation of homocysteine to methionine, also causes severe hyperhomocysteinaemia and can lead to premature

atherosclerosis and thrombotic disease (Lawrence-de-Koning *et al.*, 2003).

2.9.2.2 Nutritional deficiencies

Numerous human epidemiological studies have shown homocysteine levels correlate inversely and closely with plasma folate levels and less so with vitamin B_{12} and B_6 levels. It is obvious from the metabolism of homocysteine that when the required metabolic cofactors folic acid, vitamin B_6 (pyridoxal phosphate), or B_{12} (methyl-cobalamin) are suboptimal in the diet, homocysteine levels may elevate. It has been estimated that inadequate intake of B-vitamins and folate may account for approximately two thirds of all cases of hyperhomocysteinaemia (Lawrence-de-Koning *et al.*, 2003).

2.9.2.3 Renal failure

Normal kidney metabolism and filtration plays a prominent role in removing homocysteine from the blood: thus, hyperhomocysteinaemia is common in patients with chronic renal insufficiency and is nearly ubiquitous in patients with end-stage renal disease, who have up to a 30 times higher risk of cardiovascular-related death than the general population. Likewise, renal transplant recipients typically have elevated homocysteine levels. The causes are still not clear, but the possibilities include defective renal or extra-renal metabolism as a result of uremic toxicity. Hyperhomocysteinaemia is present in the majority of patients with chronic kidney failure. They have a plasma concentration of homocysteine elevated 3 to 4 times above normal. It is important to note that only free (unbound) homocysteine is filtered and metabolized by the kidney (Bostom & Lathrop, 1997).

2.9.2.4 Drugs That Increase Homocysteine

Hyperhomocysteinaemia can be caused by a variety of drugs, mainly those that affect vitamin levels and are related with homocysteine metabolism. Drugs such as methotrexate and phenytoin that interfere with homocysteine metabolisms (Lawrence-de-Koning *et al.*, 2003).

2.10 HOMOCYSTEINE SPECIES

Homocysteine can also oxidize with other thiols, including cysteine, with which it forms the homocysteine-cysteine mixed disulfide (Hcy-Cys) which also accounts for 5–10% of total Hcy. Less than 1 % of homocysteine exist as free Hcy (reduced Hcy). Unless otherwise mentioned, a reference to plasma homocysteine is typically referring to total plasma homocysteine. Plasma homocysteine exists in different forms including protein (mainly albumin) bound form, other oxidized forms and the free (reduced) form. The major form is the protein (mainly albumin) bound form which accounts for 70–80% of total Hcy levels in healthy individuals. Homocysteine can oxidize with itself (Hcy-Hcy) to form homocysteine dimer which accounts for 5–10% of total Hcy. (Ciaccio *et al.*, 2008; Marinou *et al.*, 2005).

2.11 DIAGNOSIS OF HOMOCYSTEINAEMIA (HOMOCYSTEINAEMIA ASSESSMENT)

The reduced homocysteine (total homocysteine) is measured either directly or after derivatization. Total homocysteine can be determined in serum or plasma by chromatographic methods or by enzyme and immunoassays. In diagnosing homocysteinaemia, plasma or serum is initially treated with a reducing agent that converts all Hcy species into the reduced form. The chromatographic assays include wide analytical range, simultaneous determination of other compounds (other sulfur aminoacids), and sometimes lower cost than commercial reagent-based assays, but they usually require skilled staff, and are labour-intensive. On the other hand, widely used enzyme and

immunoassays are usually simple to perform, and give comparable results, so they are now suitable for routine laboratories (Ciaccio *et al.*, 2008; Marinou *et al.*, 2005).

In clinical practice two methods are used for the diagnosis of hyperhomocysteinaemia. The first and simpler method is used for screening the general population for hyperhomocysteinaemia and measures the fasting or baseline levels of plasma homocysteine. The second method of diagnosing homocysteinaemia in the general population is based on the measurement of total homocysteine levels after methionine loading. Methionine loading means the ingestion of large doses of methionine (0.1 g/kg body weight) and total homocysteine is measured 2, 4 and 6 hours after the methionine is given. The post-load total homocysteine is probably more sensitive than the fasting total homocysteine due to disturbances in the transsulfuration pathway such as those caused by CBS or vitamin B_6 deficiency (Ciaccio *et al.*, 2008; Marinou *et al.*, 2005).

2.12 HOMOCYSTEINE REFERENCE RANGES

The American Heart Association released an advisory statement classifying total homocysteine plasma concentrations. The normal levels range from 5 to 15 µmol/L with a mean of 10 µmol/L. Homocysteine levels above 15µmol/L are termed hyperhomocysteinaemia. Hyperhomocysteinaemia is classified as follows: 5-15 µmol/L homocysteine as normal, 16-30 µmol/L homocysteine as moderate, 31-100 µmol/L homocysteine as intermediately elevated and total homocysteine levels above 100 µmol/L as severely elevated concentrations (Ballal *et al.*, 1997; Ciaccio *et al.*, 2008; Marinou *et al.*, 2005).

2.13 TREATMENT FOR REDUCING PLASMA HOMOCYSTEINE LEVELS

Vitamin status is a primary determinant of mild-to moderate hyperhomocysteinaemia and accounts for approximately two thirds of all such cases. Increases in homocysteinaemia are common and can easily be corrected with safe and inexpensive therapy. Folic acid and B vitamins, required for

remethylation of homocysteine to methionine, are the most important dietary determinants of homocysteine. Total homocysteine levels may be reduced by the administration of vitamins B_{12}, B_6 and folate, regardless of the levels prior to treatment. The administration of B_{12} and folate has been found to improve endothelium dependent vasodilation in the brachial artery of CAD patients, and this is probably due to the reduction in homocysteine achieved by this combined therapy. The treatment may prevent the vascular complications of homocysteinuria and the use of vitamins is likely to reduce the risk of cardiovascular disease in the general population. An increased dietary intake of fruit and vegetables, which are rich sources of folate, can contribute to reducing plasma homocysteine levels. Daily supplementation with 0.5–5.0 mg of folic acid typically lowers plasma homocysteine levels by about 25%; vitamin B_{12} supplementation of at least 0.4 mg daily further lowers levels by about 7%, and vitamin B_6 supplements may be particularly important in lowering homocysteine after methionine loading (Ciaccio *et al.*, 2008; Marinou *et al.*, 2005; Selhub & Mayer, 1999).

2.14 HYPERHOMOCYSTEINAEMIA AND HYPERTENSION: AN OVERVIEW

The mechanisms by which elevated homocysteine impairs vascular function are not completely understood. However, it has been postulated that homocysteine increases risk of cardiovascular outcome through direct toxicity to endothelial cells, production of reactive oxygen species (ROS) and consequent oxidation of low-density lipoprotein (LDL), activation of the inflammatory pathway, increased coagulability and stimulatory effects on smooth-muscle proliferation Epidemiological evidences and observational studies data suggest an association between elevated homocysteine levels and increased risk of cardiovascular complications like atherosclerosis, endothelial dysfunction, hypertension, myocardial infarction and chronic heart failure. Elevated blood homocysteine level is known to be an independent risk factor for

cardiovascular outcomes (Ankur *et al.*, 2012; Ebesunun & Obajobi, 2012; Farbstein & Levy, 2010). In order to investigate this relationship, it is important to examine the biological plausibility, as well as the mechanisms in which plasma homocysteine could possibly relate to cardiovascular disease.

2.14.1 Oxidative stress and endothelial dysfunction in hyperhomocysteinaemia

There is evidence of endothelial dysfunction with both markedly and mildly elevated homocysteine concentrations. Endothelial dysfunction is considered an early marker for atherosclerosis. Endothelial dysfunction is commonly associated with decreased nitric oxide availability. A defect in nitric oxide production or activity has been proposed as a major mechanism of endothelial dysfunction and a contributor to atherosclerosis (Davignon & Ganz, 2004; Marinou *et al.*, 2005) (Brattström & Wilcken, 2000).

Endothelial-derived nitric oxide (NO) maintains cardiovascular function by acting as a potent vasodilator; regulating vessel tone; inhibition of platelet activation and aggregation, thereby reducing platelet derived growth factor (PDGF) induced proliferation of vascular smooth muscle; and by preventing oxidative modification of low-density lipoprotein (LDL) cholesterol. The interrelations between endothelium dependent vasodilatation mediated by NO release and plasma homocysteine have been established . It has been shown that endothelium dependent vasodilatation is reduced in hyperhomocysteinaemia patients but not in their obligate heterozygote parents evidencing the probable role of hyperhomocysteinaemia in the development and progression of endothelium dysfunction (Ankur *et al.*, 2012; Farbstein & Levy, 2010).

The thiol group of homocysteine readily undergoes auto-oxidation in plasma to generate ROS, and it has been suggested that homocysteine induces cell injury/dysfunction via a mechanism involving

oxidative stress. However, this hypothesis fails to explain why cysteine, which is present in plasma at 20 to 30 fold higher concentrations than homocysteine and is more readily auto-oxidized, does not cause endothelial cell injury and is not considered a risk factor for cardiovascular disease (Lawrence-de-Koning *et al.*, 2003).

Elevated blood levels of homocysteine however down regulates nitric oxide, hence leading to cardiovascular outcomes (Farbstein & Levy, 2010). Glutathione peroxidase which prevents the oxidative inactivation of nitric oxide is also inhibited by homocysteine and thus leading to cardiovascular outcomes (Farbstein & Levy, 2010; Thambyrajah & Townend, 2000). Furthermore, homocysteine undergoes redox cycling in the presence of transition metal ions to form superoxide anion which causes oxidative damage to LDL. Oxidatively modified lowdensity lipoproteins are believed to be an important mediator of cholesterol induced atherosclerosis. The free radical superoxide anion also rapidly inactivates nitric oxide and destroys tetrahydrobiopterin, a cofactor required for NO synthesis. The superoxide anion radical which leads oxidative stress causes impaired receptor-mediated uptake of LDL and increased uptake by macrophage scavenger receptor, hence leading to atherogenesis. (Davignon & Ganz, 2004; Farbstein & Levy, 2010; Marinou *et al.*, 2005).

2.14.2 Inflammatory response and hyperhomocysteinaemia

There have been many published findings that show that plasma homocysteine enhances the production of several inflammatory cytokines**.** In general, the development and progression of atherosclerosis is considered to be a form of chronic inflammation. Human monocytes express a number of different pro-inflammatory cytokines (Austin *et al.*, 2004; Lawrence-de-Koning *et al.*, 2003). Monocyte chemo-attractant protein 1 (MCP-1) is known to enhance binding and recruitment of monocytes to the sub-endothelial cell space. MCP-1 has also been shown to increase

in cultured human endothelial and smooth muscle cells with an increased homocysteine level (Lawrence-de-Koning *et al.*, 2003). All of these cytokines, chemokines, leukocyte adhesion molecules, and haemopoetic growth factors produced during hyperhomocysteinaemia are thought to contribute to vascular inflammation and atherogenesis Furthermore, homocysteine increases the expression of IL-8, a T lymphocyte and neutrophil chemo-attractant, in cultured endothelial cells. (Austin *et al.*, 2004).

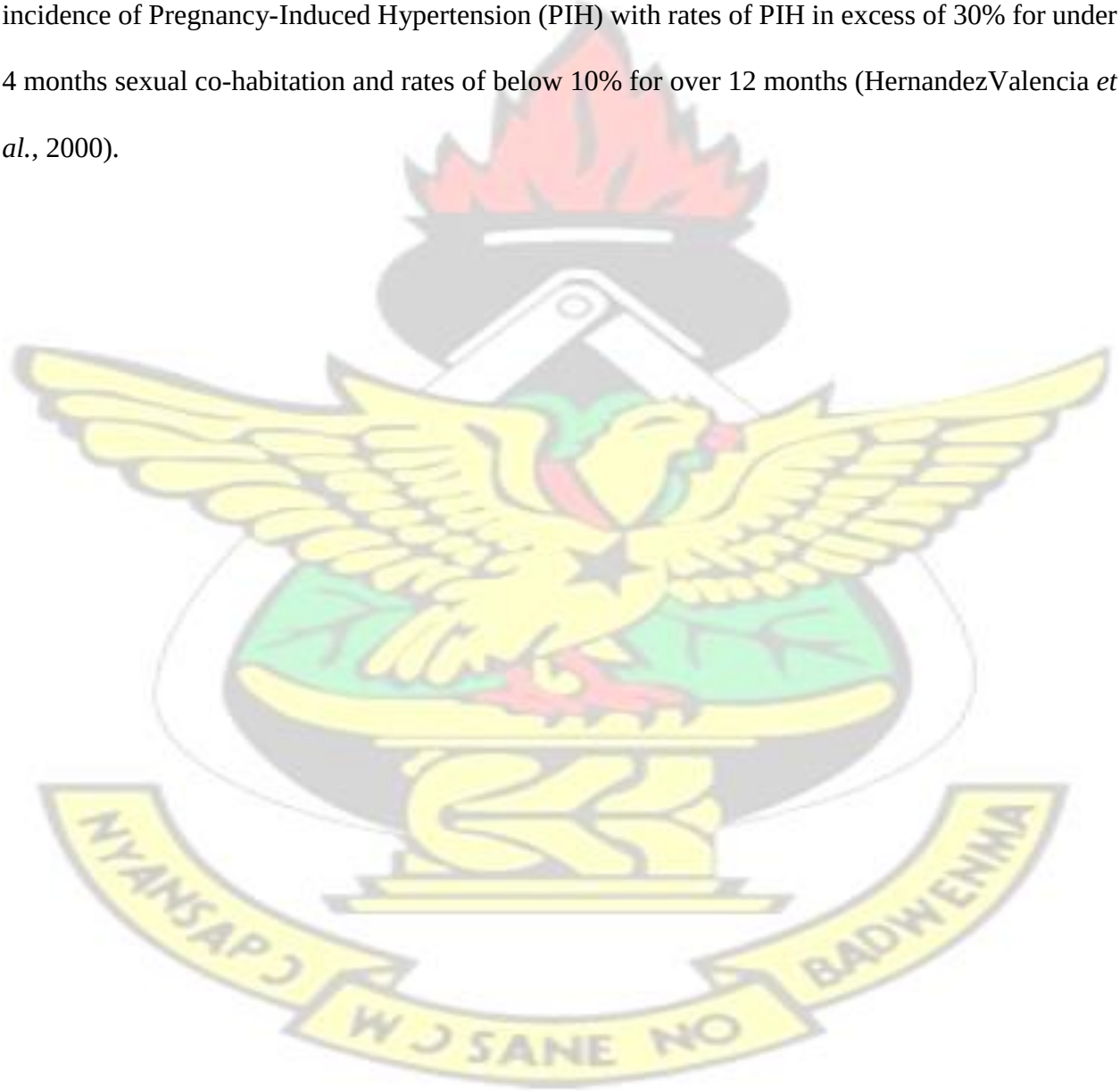
2.14.3 Atherothrombosis and hyperhomocysteinaemia

Hyperhomocysteinaemia has been found to be associated with primary thrombotic disorder affecting arteries and veins. Atherothrombotic disease is considered to be a form of chronic inflammation (Austin *et al.*, 2004). In addition, hyperhomocysteinaemia is associated with a factor or factors that primarily cause venous and arterial thrombosis. In addition, it has been reported that very high homocysteine concentrations are thrombogenic (Ankur *et al.*, 2012).

2.15 CONTRACEPTIVE USE AND PIH

Several studies have looked at the association between serum contraceptive use and PIH (Dekker, 2002; Hernandez-Valencia *et al.*, 2000). 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. 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 (Dekker, 2002; Gratacos *et al.*, 1996). Pimigravida adolescent and multigravida older women who have conceived with a new sexual partner have a greater risk of PIH, and this has been associated also with the use of barrier contraceptive methods that prevent exposure to sperm with the endometrial cavity (condoms, diaphragms, spermicides, withdrawal).

Although PIH is often considered to be purely maternal, recent data strongly indicate an important role for the male partner in the causation of this common pregnancy disorder Nonbarrier contraceptive methods or the exposure of paternal spermatic antigens are taught to be protective against development of preeclampsia (Bastani *et al.*, 2007; Hernandez-Valencia *et al.*, 2000).. An inverse relationship has been suggested between the duration of sexual co-habitation and the incidence of Pregnancy-Induced Hypertension (PIH) with rates of PIH in excess of 30% for under 4 months sexual co-habitation and rates of below 10% for over 12 months (HernandezValencia *et al.*, 2000).



Chapter 3

METHODS AND MATERIALS

3.1 STUDY DESIGN, SAMPLING TECHNIQUE AND STUDY SITE

This randomized case-control study was conducted at Comboni Hospital. Comboni Hospital is located at Fievie near Sogakofe in the South Tongu District of the Volta Region, off the AccraAflao Road. South Tongu, with Sogakope as its capital, is one of the 18 districts in the Volta Region and it is bounded in the North by the North Tongu District, in the East by Akatsi District, in the South by the Keta District and in the West by Dangbe-East District. The lower segment of the Volta River bisects it in the North-South direction while the Accra-Aflao trunk road traverses it in east-west direction. It has a population (2002) 64,852 (Ghana Districts, 2014). Population projections for 2015 based on 2010 Population Census put the current population of the district at 99,507. Services provided by this facility include: out-patient, in-patient, surgical, reproduction and child health services, x-rays, ultrasound scan, electrocardiogram, endoscopy, laboratory, mortuary, and ambulance & hearse services. The hospital also hosts specialist outreach programmes for dental and ophthalmic services. Comboni Hospital has a bed capacity of 50. During the year 2015, the total out-patient attendance was 49,864 with a total admission of 3,853. Total antenatal care (ANC) registrants was 1,115 while total ANC attendance was 6,960.

3.2 STUDY POPULATION

A total of 90 participants comprising 60 pregnancy-induced hypertensive patients (30 preeclamptic women and 30 women with gestational hypertension) and 30 healthy women with uncomplicated pregnancy (control group), aged 18-44 years were involved in this study. The study participants comprised of pregnant women in their third trimester of pregnancy (gestational age from 28 weeks to 40 weeks). The PIH patients were recruited according to their history, clinical and laboratory

findings. Healthy normotensive uncomplicated pregnant women without any pregnancy complications or any other disease condition formed the control group.

The sample size was calculated using the StatCalc utility feature of Epi Info™ 7 Statistical software (CDC, Georgia, USA). and based on sample size formula for case-control study. The following parameters were used for the sample size determination:

Two sided confidence interval = 95%,

Power (% chance of detecting) = 80%,

Ratio of cases to controls = 1:1

Hypothetical proportion of controls with exposure = 12.4% (Ahenkorah, 2009),

Hypothetical proportion of cases with exposure = 45.3%, Least

extreme Odds Ratio to be detected = 5.85.

A minimum size of 30 cases and 30 control was obtained. For the two different case groups (PE and GH) and one control group used, a total of 90 study participants at baseline (30 PE + 30 GH + 30 controls) was used.

3.2.1 Inclusion Criteria for cases

Pregnant women, both nulliparous and multiparous women aged 18 - 44 years in third trimester of pregnancy (within the gestational age of ≥ 28 - 40 weeks), singleton pregnant women, those with or without proteinuria but hypertensive (blood pressure ≥140/90 mmHg) were included as study participants. Age-matched apparently healthy pregnant women with normal blood pressure (<140/90 mmHg), absence of proteinuria, without medical and obstetrical complications certified by a consultant Obstetrician/Gynaecologist were recruited as the control group.

3.2.3 Exclusion Criteria

Pregnant women who were unable to give informed consent (learning disability, mental illness,) were excluded from the study. Participants with twin pregnancy, previous chronic hypertension, sexually transmitted infections, sickle cell anemia, diabetes mellitus, renal disease, gestational diabetes, cardiovascular disorder, malaria and use of antihypertensive medication before the recruitment were also excluded from the study.

3.3 ETHICAL CONSIDERATION

Informed consent was obtained from participants before enrollment and the study protocol was approved by the ethics committee of Comboni hospital.

3.4 QUESTIONNAIRE

A well-structured pre-tested questionnaire was used to obtain detailed medical, obstetric, dietary and socio-demographic data from each participant.

3.5 MEASUREMENT OF BLOOD PRESSURE

In accordance with recommendations of the American Heart Association (Kirkendall *et al.*, 1967), the blood pressure of each participant was measured by trained personnel using a mercury sphygmomanometer (Omron M2 basic, Netherland). The procedure was repeated for each patient between 5-10 minutes. Mean values of duplicate measurements were recorded as the blood pressure to the nearest 2.0 mmHg.

3.6 ANTHROPOMETRIC MEASUREMENTS

Patients were made to stand without their sandals, bags or anything of significant weight on the weighing scale (Jactermac RGZ-160, Germany) and against the stadiometer (Jactermac RGZ160, Germany). The weight was read to the nearest 0.1 kilogram and recorded. The value for the height

was recorded to the nearest 0.1 centimeter and then converted to meters. Body mass index (BMI)was calculated as the ratio of body weight in kilograms to height in square meter.

3.7 BLOOD SAMPLE COLLECTION

A volume of 5 ml of venous blood samples was drawn from the antecubital vein of each participant after an overnight fast (8-12 h) and immediately transferred into gel containing tubes. The clotted sample will be centrifuged at 3000 rpm for 5 minutes and the serum stored at -80°C until assayed.

3.7.1 Biochemical analysis

After thawing, the homocysteine, folate, vitamin B_{12}, and lipid profile (HDL-C, LDL-C, total cholesterol and triglycerides) concentrations of each blood sample was analyzed using appropriate techniques.

3.7.1.1 Determination of serum homocysteine

Serum homocysteine (Hcy) was determined by enzymatic UV, using Homocysteine liquiUV diagnostics kit, Germany. First, the oxidized form of homocysteine was reduced by tris [2carboxyethyl] phosphine (TCEP) and reacts afterwards with serine, catalyzed by cystathione beta-synthase (CBS) to form cystathione. Cystathione was then broken down by cystathione beta-lyase (CBL) to homocysteine, pyruvate and ammonia. Pyruvate was then converted by lactate dehydrogenase (LDH) to lactate with NADH.

$$R - SS - Hcy \xrightarrow{TCEP} Hcy$$

$$Hcy + Serine \xrightarrow{CBS} Cystathione$$

$$Cystathione + NADH + H^+ \xrightarrow{CBL} Hcy + Pyruvate + Ammonia + NAD^+$$

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

R = dimerised homocysteine, thiol residue or protein

The rate of NADH conversion to NAD^+ is directly proportional to the homocysteine concentration measured at 340 nm.

Reagent preparation

The reagent was ready to use.

Calculation

$$Homocystein\left(\frac{\mu mol}{L}\right) = \frac{Absorbance\ of\ Test}{Absorbance\ of\ Standard} \times Concentration\ of\ standard\ (\mu mol/L)$$

3.7.1.2 Determination of serum Vitamin B_{12} concentration

The serum concentration of vitamin B_{12} was determined using the AccuBind ELISA microwells (Monobind Inc., Lake Forest, CA 92630, USA).

Serum vitamin B_{12} was determined using the Delayed Competitive Enzyme Immunoassay. The essential reagents required for an enzyme immunoassay include antibody, enzyme-antigen conjugate and native antigen. Upon mixing the biotinylated antibody with a serum containing the antigen, a reaction results between the antigen and the antibody. The reaction is illustrated by the following equation:

$$Ag + Ab_{Btn} \leftrightarrow AgAb_{Btn}$$

Ab_{Btn} = Biotinylated antibody
Ag = antigen (Variable Quantity)

$AgAb_{Btn}$ = Immune Complex

After a short incubation, the enzyme conjugate was added (this delayed addition permits an increase in sensitivity for low concentration samples). Upon the addition of the enzyme conjugate, competition reaction results between the enzyme analog and the antigen in the sample for a limited number of antibody binding sites (not consumed in the first incubation).

$$(Enz)Ag + Ag + rAb_{Btn} \overset{k_a}{\leftrightarrow} {}_{k_{-a}} AgAb_{Btn} + (Enz)AgAb_{Btn}$$

(Enz)Ag = Enzyme antigen conjugate (constant quantity)

(Enz)$AgAb_{Btn}$ = Enzyme antigen conjugate – antibody complex rAb_{Btn}

= Biotinylated antibody not reacted in first reaction

K_a = Rate constant of association

K_{-a} = Rate constant of dissociation

$K = K_a / K_{-a}$ = Equilibrium constant

A simultaneous reaction between the biotin attached to the antibody and the streptavidin immobilized on the microwell occurs. This effects the separation of the antibody bound fraction after decantation or aspiration.

$$AgAb_{Btn} + (Enz)AgAb_{Btn\_} + strptavidin_{cw} \rightarrow immobilized\ complex$$

$Streptavidin_{cw}$ = streptavidin immobilized on well

Immobilized complex = sandwich complex bound to the solid surface.

The enzyme activity in the antibody bound fraction is inversely proportional to the native antigen concentration.

Reagent preparation

All kit components and samples were brought to room temperature (18-25^{o}C) before use.

Wash buffer: The wash buffer was prepared by diluting the content of the wash solution to 1000 ml.

Extraction agent: The extraction agent was also prepared using a 1 in 40 dilution. This was done by adding 1 part of the stabilizing agent to 40 parts of the releasing agent.

Sample extraction: To prepare each specimen and standard reagent for use, 100 µL of each sample was dispensed into individual test tubes. 50 µL of the prepared extraction agent was added to each test tube containing the samples, shaking after each addition. After the reaction was allowed to proceed for 15 minutes, 50 µL of a neutralizing buffer was added to each test tube and then vortexed. After the mixing, the reaction was allowed to go to completion by waiting for 5 minutes before dispensing into the microwells.

Assay procedure

50 µL of the appropriate extracted vitamin B_{12} standard reagents (six standard reagents with different concentrations) and specimen were pipetted into the assigned well, after which 50 µL of vitamin B_{12} biotin reagent was added to all the wells. The content of the microplate were then gently mixed for about 20 – 30 seconds. After covering the microplate, it was incubated for 45 minutes at room temperature. Following the incubation, 50 µL of the vitamin B_{12} enzyme reagent was gently added to all the wells, and the content of the microplate was again gently mixed for

about 20 – 30 seconds. After the microplate was covered and incubated for 30 minutes at room temperature, its content was decanted and the plate rapidly blotted to dry with an absorbent paper. The microplate was washed manually by adding 350 µL of wash buffer to each well, and then followed by decanting and blotting to remove residual water. The washing procedure was repeated two more times. 100 µL of substrate reagent was then added to all the wells, with strict adherence to manufacturer's instructions. The microplate was then incubated at room temperature for 20 minutes. The reaction was stopped after adding 50 µL of the stop solution to each well and gently mixing for 15 – 20 seconds. Absorbances were read at 450nm within 15 minute after adding stop solution using microplate ELISA reader (Mindray MR-96A; Shenzhen Mindray Bio-medical electronics Co., Ltd, China). By utilizing several different serum references of known antigen concentration, a dose response curve was generated from which the antigen concentration of an unknown was ascertained.

3.7.1.3 Determination of serum folate concentration

The serum concentration of folate was determined using the AccuBind ELISA microwells (Monobind Inc., Lake Forest, CA 92630, USA).

Serum folate was determined using the competitive binding protein assay. The essential reagents required for the competitive binding assay include specific binding protein, enzyme-antigen conjugate and native antigen. Upon mixing the enzyme-antigen conjugate, biotinylated binding protein and a serum containing the native antigen, a competition results between the native antigen and enzyme-antigen conjugate for a limited number of binding sites. The interaction is illustrated by the following equation:

$$(Enz)Ag + Ag + BP_{Btn} \overset{k_a}{\underset{k_{-a}}{\leftrightarrow}} AgBP_{Btn} + (Enz)AgBP_{Btn}$$

BP_{Btn} = Biotinylated binding protein

Ag = native antigen (variable quantity)

(Enz)Ag = Enzyme antigen conjugate (constant quantity)

$AgBP_{Btn}$ = antigen binding protein complex

$(Enz)AgBP_{Btn}$ = Enzyme antigen binding protein complex

K_a = Rate constant of association

K_{-a} = Rate constant of dissociation

$K = K_a / K_{-a}$ = Equilibrium constant

A simultaneous reaction between the biotin attached to the binding protein and the streptavidin immobilized on the microwell occurs. This effects the separation of the binding protein enzyme bound fraction after decantation or aspiration.

$$AgAb_{Btn} + (Enz)AgAb_{Btn\_} + strptavidin_{cw} \rightarrow immobilized\ complex$$

$Streptavidin_{cw}$ = streptavidin immobilized on well

Immobilized complex = sandwich complex bound to the solid surface.

The enzyme activity in the binding protein bound fraction is inversely proportional to the native antigen concentration.

Reagent preparation

All kit components and samples were brought to room temperature (18-25^{o}C) before use.

Wash buffer: The wash buffer was prepared by diluting the content of the wash solution to 1000 ml.

Extraction agent: The extraction agent was also prepared using a 1 in 40 dilution. This was done by adding 1 part of the stabilizing agent to 40 parts of the releasing agent.

Sample extraction: To prepare each specimen and standard reagent for use, 100 µL of each sample was dispensed into individual test tubes. 50 µL of the prepared extraction agent was added to each test tube containing the samples, shaking after each addition. After the reaction was allowed to proceed for 15 minutes, 50 µL of a neutralizing buffer was added to each test tube and then vortexed. After the mixing, the reaction was allowed to go to completion by waiting for 5 minutes before dispensing into the microwells.

Assay procedure

50 µL of the appropriate extracted folate standard reagents (six standard reagents with different concentrations) and specimen were pipetted into the assigned well, after which 50 µL of folate enzyme reagent was added to all the wells. The content of the microplate were then gently mixed for about 20 – 30 seconds. 50 µL of folate biotin reagent was then gently added to all the wells, and the content of the microplate was again gently mixed for about 20 – 30 seconds. After the microplate was covered and incubated for 45 minutes at room temperature, its content was decanted and the plate rapidly blotted to dry with an absorbent paper. The microplate was washed manually by adding 350 µL of wash buffer to each well, and then followed by decanting and blotting to remove residual water. The washing procedure was repeated two more times. 100 µL of substrate reagent was then added to all the wells. The microplate was then incubated at room temperature for 20 minutes. The reaction was stopped after adding 50 µL of the stop solution to

each well and gently mixing for 15 – 20 seconds. Absorbances were read at 450 nm within 15 minute after adding stop solution using microplate ELISA reader (Human Plus, Germany). By utilizing several different serum references of known antigen concentration, a dose response curve was generated from which the antigen concentration of an unknown was ascertained. By utilizing several different serum references of known antigen concentration, a dose response curve was generated from which the antigen concentration of an unknown was ascertained

3.7.1.4 Determination of serum cholesterol

Serum cholesterol was determined by cholesterol oxidase (COD)/POD method. Cholesterol esters are broken down to cholesterol and fatty acids. The cholesterol is then oxidized to chole-4en-3-one and hydrogen peroxide. The hydrogen peroxide is the hydrolyzed by a peroxidase to a red dye (quinoneimine).

$$\text{Cholesterol esters} + H_2O \xrightarrow{\textit{Cholesterol esterase}} \text{Cholesterol} + \text{Fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\textit{Cholesterol oxidase}} \text{Cholest} - 4 - \text{en} - 3 - \text{one} + H_2O_2$$

$$2H_2O_2 + Hydroxybenzoicacid + 4 - aminoantipyrine + Phenol \xrightarrow{\textit{Peroxidase}} Quinoneamine + 4H_2O$$

The intensity of chromogen (Quinoneimine) formed is directly proportional to the Cholesterol concentration.

Reagent preparation

The reagent was ready to use.

Calculation

$$Cholesterol\ (\frac{mmol}{L}) = \frac{Absorbance\ of\ Test}{Absorbance\ of\ Standard} = Concentration\ of\ standard\ (mmol/L)$$

3.7.1.5 Determination of serum triglycerides

Serum triglyceride was determined by Glycerol phosphate oxidase/peroxidase method. The glycerol is then phosphorylated by ATP to glycerol-3-phosphate (G-3-P) and ADP in a reaction catalyzed by glycerol kinase. G3P 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-dichloro-2-hydroxybenzen (3, 5DHBS) in a reaction catalyzed by peroxidase to yield a red coloured quinoneimine dye.

$$Triglyceride + H_2O \xrightarrow{Lipase} Glycerol + Fatty\ acids$$

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

$$G3P + O_2 \xrightarrow{Glycerolphosphate\ oxidase} DAP\ H_2O_2$$

$$H_2O_2 + 4 - AAP + 3{,}5 - DHBS \xrightarrow{Peroxidase} Quinoneamine + H_2O$$

The intensity of the colour produced is directly proportional to the concentration of triglycerides in the sample.

Reagent preparation

The reagent was ready to use.

Calculation

$$Cholesterol\left(\frac{mmol}{L}\right) = \frac{Absorbance\ of\ Test}{Absorbance\ of\ Standard} \times Concentration\ of\ standard\ (mmol/L)$$

3.7.1.6 Determination of serum high density lipoprotein cholesterol (HDL-C)

HDL-C was determined by precipitating method. The first reagent contains anti human βlipoprotein antibody which bind 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 intensity of the red colour produced is directly proportional to the HDLcholesterol in the sample when read at 500 nm.

3.7.1.7 Determination of serum low density lipoproteins cholesterol (LDL-C)

The LDL-Cholesterol concentration (LDL-C) is calculated from the total cholesterol concentration (TC), HDL-Cholesterol concentration (HDL-C) and the triglycerides (TG) concentration according to Friedewald"s equation found below:

$$LDL = Total\ cholesterol - HDL - \frac{Triglycerides}{2.2}\ mmol/L$$

3.7.1.8 Urine collection and determination of proteinuria

About 10-20 ml of freshly voided early morning urine was collected from each participant provided into clean, wide mouth and leak proof plastic containers. Qualitative proteinuria was immediately

assessed using dipstick (CYBOW™ DFI Co. Ltd., Gimhae-City, Republic of Korea). Proteinuria was considered as the presence of urinary protein in concentrations more than 2+ on the urine dipstick.

3.8 DETERMINATION OF IUGR AND BIRTH WEIGHT

The estimated foetal weight and the birth weight (grams) were obtained from midwives and the medical record of each participant. Using the smoothed percentiles of estimated birth weight (grams) for gestational age (Table 2.1), intrauterine growth restriction (IUGR) was determined using the 10^{th} percentile.

3.9 STATISTICAL ANALYSIS

IBM SPSS Statistics for Windows, Version 21.0 (Armonk, NY: IBM Corporation) and Microsoft Excel 2013 were used for the statistical analysis. Continuous variables were expressed as mean ± standard deviation, while categorical variables were expressed as proportion. The independent sample t-test, chi square and Fisher exact tests were used where appropriate to compare means of the women with PIH (preeclampsia and gestational hypertension separately and combined) against the control group. Pearson correlation coefficient test was used to determine the correlation between study parameters. Using a multivariate logistic regression with a 95% confidence interval (CI), was the odds ratio (OR) was determined to quantify the risk of women with PIH in comparison with controls. Statistical significance level was set at $P< 0.05$.

Chapter 4

RESULTS 4.1 SOCIODEMOGRAPHIC AND CLINICAL CHARACTERISTICS OF STUDY

PARTICIPANTS

A total of 90 participants comprising 60 pregnancy induced-hypertensive patients (30 preeclamptic and 30 gestational hypertensive pregnant women) and 30 normal pregnant women within the age group of 19 years to 44 years were included in this study from January 2015 to December 2015. The hypertensive pregnant women and the control group presented with similar age. Majority of the study participants were within the age range of 25-34 years (37.8% and 30.0% for the age group 25-29 years and 30-34 years respectively). Other age groups percentages were as follows: 15.6%, 11.1%, 3.3% and 2.2% for 35-39 years, 20-24 years, >40 years and < 20 years, respectively. Thirty percent (30%) of the participants had completed Junior High School whereas 22.2%, 18.9% and 13.3% had completed Senior High School, Tertiary, and Basic (Primary) education, respectively. About 40% (41.1%) of the pregnant women were obese, whereas 36.7% and 22.2% were overweight and normal weight, respectively. About 50% (52.2%) of the study participants had normal systolic blood pressure (SBP), whereas 47.8% had high SBP. 61.1% and 38.9% of the pregnant women had normal and high diastolic blood pressure (DBP), respectively. The difference in the SBP and DBP of the PIH, PE and GH patients on comparison with the normal pregnant women was statistically significant at $P<0.05$ (Table 4.1).

Table 4.1: Sociodemographic and clinical characteristics of study Participants

Parameters	PIH (n=60) PE + GH	PE (n=30)	GH (n=30)	NP (n=30)	Total (n=90) PE + GH + NP
Age (yrs)	30.17 ± 0.67	30.50 ± 1.06	29.83 ± 0.82	29.13 ± 0.91	29.82 ± 0.93
Age category (yrs)					
<20	1 (1.7%)	1 (3.3%)	Nil (0.0%)	1 (3.3%)	2 (2.2%)
20-24	6 (10.0%)	2 (6.7%)	4 (13.3%)	4 (13.3%)	10 (11.1%)
25-29	17 (28.3%)	9 (30.0%)	8 (26.7%)	10 (33.3%)	27 (30.0%)
30-34	24 (40.0%)	8 (26.7%)	16 (53.3%)	10 (33.3%)	34 (37.8%)
35-39	9 (15.0%)	8 (26.7%)	1 (3.3%)	5 (16.7%)	14 (15.6%)
≥40	3 (5.0%)	2 (6.7%)	1 (3.3%)	Nil (0.0%)	3 (3.3%)
Educational Level					
No Education	11 (18.3%)	8 (26.7%)	3 (10.0%)	3 (10.0%)	14 (15.6%)
Basic	10 (16.7%)	6 (20.0%)	4 (13.3%)	2 (6.7%)	12 (13.3%)
JHS	18 (30.0%)	8 (26.7%	10 (33.3%)	9 (30.0%)	27 (30.0%)
SHS	13 (21.7%)	5 (16.7%)	8 (26.7%)	7 (23.3%)	20 (22.2%)
Tertiary	8 (13.3%)	3 (10.0%)	5 (16.7%)	9 (30.0%)	17 (18.9%)
BMI					
Normal	11 (18.3%)	7 (23.3%)	4 (13.3%)	9 (30.0%)	20 (22.2%)
Overweight	20 (33.3%)	8 (26.7%)	12 (40.0%)	13 (43.3%)	33 (36.7%)
Obese	29 (48.3%)	15 (50.0%)	14 (46.7%)	8 (26.7%)	37 (41.1%)
SBP					
Normal	17 (28.3%)*	5 (16.7%)*	12 (40.0%)*	30 (100.0%)	47 (52.2%)
High	43 (71.7%)	25 (83.3%)	18 (60.0%)	Nil (0.0%)	43 (47.8%)
DBP					
Normal	25 (41.7%)*	11 (36.7%)*	14 (46.7%)*	30 (100.0%)	55 (61.1%)
High	35 (58.3%)	19 (63.3%)	16 (53.3%)	Nil (0.0%)	35 (38.9%)

*Chi-square or Fischer exact test whenever n < 5 was used to compare between each groups (PIH, GH and PE) to NP group. *p<0.05 was considered a statistically significant difference. BMI of 19 Kg/m^2 – 24.9 Kg/m^2, 25.0 Kg/m^2 – 29.9 Kg/m^2 and >30 Kg/m^2 were categorized as normal, overweight and obese respectively PIH: Pregnancy school; SHS: Senior high school; BMI: Body Mass Index; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure.*

4.2 THE CLINICAL VARIABLES OF THE STUDY POPULATION.

The clinical variables of the study population are shown in Table 4.2. The mean age of the PIH cases (PE and GH) was 30.17 ± 0.67 years; (30.50 ± 1.06 years for the preeclamptic patients and 29.83 ± 0.82 years for the gestational hypertensive patients) and 29.13 ± 0.91 years for women with normal pregnancy. There was no statistically significant difference in the mean age between the PIH, PE and GH patients in comparison with women with normal pregnancy. SBP, DBP and homocysteine levels were significantly higher ($P<0.05$) in the PIH, GH, and PE patients than in the group of women with normal pregnancy. It was only in the GH patients that the gestational age was significantly different in comparison with women with normal pregnancy. Although the body mass index (BMI) was higher in the PIH, PE and GH patients when compared with the normal pregnant women, it was only in the PIH patients that there existed a statistically significant difference in comparison with the controls. The infant birthweight of the PIH, PE and GH patients were significantly lower when compared with women with normal pregnancy. Although the estimated foetal weight was lower in the PIH, PE and GH patients than the group with normal pregnancy, it was only in the PIH and PE patients that the difference was significant. In general, the total cholesterol, triglycerides and LDL-C levels were higher in PIH, PE and GH patients than in the group with normal pregnancy. However, it was only triglycerides and the LDL-C of the PIH and PE patients that showed statistically significant differences ($P<0.05$) in comparison with the group with normal pregnancy. Although the HDL-C was lower in the PIH, PE and GH patients when compared with the group with normal pregnancy, it was only the PE patients that showed a statistically significant difference ($P<0.05$). Levels of vitamin B_{12} and folate were lower in the PIH, PE and GH patients in comparison with the normal pregnancy group.

Vitamin B_{12} and folate levels in the PIH and PE patients showed statistically significant differences when compared with the normal pregnancy group ($P<0.05$).

Table 4.2: The Clinical Variables of the Study Population.

Parameters	PIH (n=60)	‡p-value	PE (n=30)	♀p-value	GH (n=30)	φp-value	NP (n=30)
Age (years)	30.17 ± 5.15	0.367	30.50 ± 5.78	0.330	29.83 ± 4.51	0.571	29.13 ± 4.98
Gestational Age (yrs)	35.45 ± 2.53	0.127	34.50 ± 2.83	0.834	36.40 ± 1.80	0.001	34.63 ± 2.00
Weight (Kg)	83.32 ± 17.81	0.003	84.07± 21.33	0.011	82.57 ± 13.76	0.004	72.20 ± 12.69
Height m	1.60 ± 0.08	0.034	1.62 ± 0.09	0.029	1.59 ± 0.08	0.212	1.56 ± 0.12
BMI (Kg/m2)	30.04 ± 4.85	0.043	30.35 ± 5.71	0.063	29.73 ± 3.88	0.083	27.93 ± 4.02
SBP (mmHg)	152.50 ± 13.51	<0.0001	155.67 ± 15.30	<0.0001	149.33 ± 10.81	<0.0001	112.33 ± 10.06
DBP (mmHg)	98.00 ± 9.17	<0.0001	99.33 ± 10.81	<0.0001	96.67 ± 7.11	<0.0001	73.67 ± 9.64
EFW (Kg)	2.48 ± 0.79	<0.0001	2.00 ± 0 .62	<0.0001	2.95 ± 0.65	0.772	3.00 ± 0.40
BWT (Kg)	2.81 ± 0.54	<0.0001	2.50 ± 0.36	<0.0001	3.12 ± 0.52	0.022	3.46 ± 0.62
TC (mmol/L)	4.28 ± 1.47	0.053	4.17 ± 1.48	0.162	4.38 ± 1.47	0.053	3.64 ± 1.45
TRG (mmol/L)	1.23 ± 0.90	0.006	1.24 ± 0.84	0.024	1.20 ± 0.97	0.070	0.85 ± 0.33
LDL (mmol/L)	2.85 ± 1.46	0.010	2.92 ± 1.54	0.026	2.78 ± 1.41	0.054	2.15 ± 1.03
HDL (mmol/L)	1.07 ± 0.57	0.057	0.87 ± 0.50	0.001	1.28 ± 0.57	0.643	1.31 ± 0.46
Homocysteine (µmol/L)	23.13 ± 12.53	<0.0001	32.35 ± 11.08	<0.0001	13.91 ± 4.57	<0.0001	9.32 ± 2.73
Vitamin B_{12} (pg/mL)	515.57 ± 205.23	0.011	483.44 ± 171.02	0.003	547.69 ± 233.03	0.078	665.26 ± 273.19
Folate (ng/mL)	10.65 ± 9.08	0.006	8.21 ± 6.32	<0.0001	13.09 ± 10.74	0.100	18.18 ± 12.80

PIH: pregnancy-induced hypertension; PE: preeclampsia; GH: gestational hypertension; NP: normal pregnant control; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; TC: total serum cholesterol; TRG: triglycerides; LDL: low density lipoprotein cholesterol; HDL: high density lipoprotein cholesterol. Data was expressed as mean ± standard deviation (SD). Independent (unpaired) t-test was to compare means of the clinical variables with the normal pregnant women. Each comparison is

performed between hypertensive groups individually (GH-gestational hypertension, PE- preeclampsia, or PIH-gestationahypertension + preeclampsia combined) and the normal pregnant women. ‡p-value (PIH vs NP); ♀p-value (PE vsNP); φp-value (GH vsNP).

4.3 OBSTETRIC CHARACTERISTICS OF STUDIED PARTICIPANTS

Table 4.3 shows the obstetric characteristics of the study participants. Majority (66.7%) of the pregnant women were within 34 - 37 weeks of gestation. The highest percentage (30.0%) of participants was primiparous, whereas 26.7%, 22.2%, 13.3% and 7.8% were secundiparous, tertiparous and multiparous, respectively. Majority (30.0%) of the participants were multigravida. About sixty-four percent (64.4%) of the pregnant women had never used any contraceptive prior to the pregnancy; whereas 34.4% and 1.1% once used Depo-Provera and Norplant, respectively. Of the pregnant women who used Depo-Provera, majority (77.4%) were preeclamptic pregnant women. 91.1% of the study participants had no family history of hypertension. Majority of the pregnant women with family history of hypertension were amongst the preeclamptic patients. There were no significant differences in the previous obstetric history

(abortion, induced abortion, spontaneous abortion, miscarriage and still birth) of the PIH, PE and GH participants on comparison with the normal pregnant women.

Table 4.3: Obstetric characteristics of studied participants

Parameters	PIH (n=60) PE + GH	PE (n=30)	GH (n=30)	NP (n=30)	Total (n=90) PE + GH + NP
Gestational Age at sampling					
<34 weeks	11 (18.3%)	9 (30.0%)	2 (6.7%)*	8 (26.7%)	19 (21.1%)
34-37 weeks	39 (65.0%)	18 (60.0%)	21 (70.0%)	21 (70.0%)	60 (66.7%)
≥38 weeks	10 (16.7%)	3 (10.0%)	7 (23.3%)	1 (3.3%)	11 (12.2%)
Parity					
Nulliparous	14 (23.3%)	10 (33.3%)	4 (13.3%)	6 (20.0%)	20 (22.2%)
Primipara	16 (26.7%)	6 (20.0%)	10 (33.3%)	11 (36.7%)	27 (30.0%)
Secundipara	15 (25.0%)	4 (13.3%)	11 (36.7%)	9 (30.0%)	24 (26.7%)
Tertipara	9 (15.0%)	6 (20.0%)	3 (10.0%)	3 (10.0%)	12 (13.3%)
Multipara (≥4)	6 (10.0%)	4 (13.3%)	2 (6.7%)	1 (3.3%)	7 (7.8%)
Gravidity					
Primigravida	8 (13.3%)	6 (20.0%)	2 (6.7%)	5 (16.7%)	13 (14.4%)
Secungravida	16 (26.7%)	7 (23.3%)	9 (30.0%)	10 (33.3%)	26 (28.9%)
Tertigravida	14 (23.3%)	4 (13.3%)	10 (33.3%)	10 (33.3%)	24 (26.7%)
Multigravida (≥4)	22 (36.7%)	13 (43.3%)	9 (30.0%)	5 (16.7%)	27 (30.0%)
Contraceptive use					
Yes	30 (50%)	24 (80%)	6 (20%)	2 (6.7%)	32 (35.6%)
No	30 (50%)	6 (20%)	24 (80%)	28 (93.3%)	58 (64.4%)
Type of contraceptive used					
Depo-Provera	29 (48.3%)*	24 (80.0%)*	5 (16.7%)	2 (6.7%)	31 (34.4%)
Norplant	1 (1.7%)	Nil (0.0%)	1 (3.3%)	Nil (0.0%)	1 (1.1%)
No contraceptive used	30 (50.0%)	6 (20.0%)	24 (80.0%)	28 (93.3%)	58 (64.4%)
Family history of HTN					
Yes	6 (10.0%)	5 (16.7%)	1 (3.3%)	2 (6.7%)	8 (8.9%)
No	54(90.0%)	25 (83.3%)	29 (96.7%)	28 (93.3%)	82 (91.1%)
Previous obstetric history No complication	46 (76.7%)	23 (76.7%)	23 (76.7%)	25 (83.3%)	71 (78.9%)
Induced abortion	4 (6.7%)	2 (6.7%)	2 (6.7%)	1 (3.3%)	5 (5.6%)
Spontaneous abortion	6 (10.0%)	4 (13.3%)	2 (6.7%)	3 (10.0%)	9 (10.0%)
Miscarriage	3 (5.0%)	1 (3.3%)	2 (6.7%)	1 (3.3%)	4 (4.4%)
Still birth	1 (1.7%)	Nil (0.0%)	1 (3.3%)	Nil (0.0%)	1 (1.1%)

*Chi-square or Fischer exact test whenever n < 5 was used to compare between each groups (PIH, GH and PE) to NP group. *p<0.05 was considered a statistically significant difference. PIH: Pregnancy Induced Hypertensive; PE: Preeclampsia; GH: Gestational hypertension; NP: Normal pregnant control; HTN: Hypertension.*

4.4 FOOD INTAKE OF THE STUDY POPULATION

Table 4.4 shows the food intake of the study population. The frequency of meat consumption by the pregnant women was 46.7%, 36.7% and 8.9% once a week, twice a week and daily, respectively. Non-meat consumers constituted 7.8% of the study participant. All the study participants consumed fish as part of their diet. The frequency of fish consumption was 93.3%, 3.3% and 3.3% daily, twice a week and once a week, respectively. Green leafy vegetables were consumed by the pregnant women as follows: 42.2%, 32.2% and 18.9% twice a week, once a week and daily, respectively. However, 6.7% of the study participants do not eat green leafy vegetables at all. Of the nutritional data of the study population, it was only in the PIH and GH patients that consumption of green leafy vegetables showed a statistically significant difference ($P<0.05$) when compared with the control group.

Table 4.4: Nutritional data of the study population

Parameters	PIH (n=60) PE + GH	PE (n=30)	GH (n=30)	NP (n=30)	Total (n=90) PE + GH + NP
Meat intake None					
	6 (10.0%)	4 (13.3%)	2 (6.7%)	1 (3.3%)	7 (7.8%)
Daily	6 (10.0%)	4 (13.3%)	2 (6.7%)	2 (6.7%)	8 (8.9%)
Twice a week	20 (33.3%)	8 (26.7%)	12 (40.0%)	13 (43.3%)	33 (36.7%)
Once a week	28 (46.7%)	14 (46.7%)	14 (46.7%)	14 (46.7%)	42 (46.7%)
Fish intake					
None	Nil (0.0%)	Nil (0.0%)	Nil (0.0%)	Nil (0.0%)	Nil (0.0%)
Daily	54 (90.0%)	25 (83.3%)	29 (96.7%)	30 (100.0%)	84 (93.3%)
Twice a week	3 (5.0%)	3 (10.0%)	Nil (0.0%)	Nil (0.0%)	3 (3.3%)
Once a week	3 (5.0%)	2 (6.7%)	1 (3.3%)	Nil (0.0%)	3 (3.3%)
Green leafy vegetables					
None	4 (6.7%)*	4 (13.3%)	Nil (0.0%)*	2 (6.7%)	6 (6.7%)
Daily	9 (15.0%)	2 (6.7%)	7 (23.3%)	8 (26.7%)	17 (18.9%)
Twice a week	22 (36.7%)	16 (53.3%)	6 (20.0%)	16 (53.3%)	38 (42.2%)
Once a week	25 (41.7%)	8 (26.7%)	17 (56.7%)	4 (13.3%)	29 (32.2%)

PIH: pregnancy-induced hypertension; PE: preeclampsia; GH: gestational hypertension; NP: Normal pregnant control.

4.5 PREGNANCY OUTCOMES IN THE STUDY PARTICIPANT

Table 4.5 shows the foetal growth restriction and birthweight among the study participants. Intrauterine growth restriction (IUGR) was present if the estimated foetal weight (EFW) was below the 10th percentile for their gestational age. Growth restriction was demonstrated in 23.3% of the pregnant women, with the majority in the preeclamptic patients. Pregnant women in the control group showed no foetal growth restriction. Babies who weigh below 2.5 Kg at birth are considered as underweight babies while those weighing 2.5 Kg or more at birth are considered as normal weight. Under-weight babies were associated with 18.9% of the pregnancies, whereas 81.1% delivered babies that were of normal weight. Majority of the underweight babies came from preeclamptic women.

Table 4.5: Pregnancy outcomes in the study participants

Parameters	PIH (n=60) PE + GH	PE (n=30)	GH (n=30)	NP (n=30)	Total (n=90) PE + GH + NP
Foetal growth restriction					
Yes	21 (35.0%)	15 (50.0%)	6 (20.0%)	Nil (0.0%)	21 (23.3%)
No	39 (65.0%)	15 (50.0%)	24 (80.0%)	30 (100.0%)	69 (76.7%)
Birth weight					
Normal weight	43 (71.7%)	16 (53.3%)	27 (90.0%)	30 (100.0%)	73 (81.1%)
Underweight	17 (28.3%)	14 (46.7%)	3 (10.0%)	Nil (0.0%)	17 (18.9%)

PIH: pregnancy-induced hypertension; PE: preeclampsia; GH: gestational hypertension; NP: Normal pregnant control.

4.6 MULTIVARIATE LOGISTIC REGRESSION OF FACTORS ASSOCIATED WITH PIH, PE AND GH

Table 4.6 shows the multivariate logistic regression of the risk factors associated with PIH, PE and GH. This study observed that the use of contraceptives prior to pregnancy was associated with 26-fold increase in the odds of developing preeclampsia. More so, the use of the contraceptive Depo-Provera is associated with about 30-fold increase in the odds of developing preeclampsia.

Table 4.6: Multivariate logistic regression of factors associated with PIH, PE and GH

Variable	PIH		PE		GH	
	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value
Contraceptive use Yes						
	0.07 (0.02-0.33)	0.001	26.00 (8.12-83.25)	<0.001	0.33 (0.12-0.92)	0.033
No*	1.00	-	1.00	-	1.00	-
Type of contraceptive used				-		-
None*	1.00	-	1.00		1.00	
Depo-Provera	0.07 (0.02-0.34)	0.001	29.71 (9.01-97.96)	<0.001	0.27 (0.09-0.81)	
Norplant	0.00	1.000	-	-	0.23 (1.00)	-

*PIH: Pregnancy Induced Hypertensive; PE: Preeclampsia; GH: Gestational hypertension; OR: Odds Ratio; CI: Confidence Interval; HTN: Hypertension, *Reference group.*

4.7 PEARSON CORRELATION BETWEEN THE STUDIED CLINICAL VARIABLES IN PIH AND NP

Table 4.7 shows the correlation between the studied parameters in pregnancy induced hypertensive patients and normal pregnant women.

In the PIH patients, homocysteine showed a statistically significant negative correlation with estimated foetal weight, infant birthweight, folate, gestational age and HDL-C. Vitamin B_{12}, LDL-C and total cholesterol also showed a negative but not significant correlation with homocysteine. However, systolic blood pressure, diastolic blood pressure and triglycerides showed statistically significant positive correlation with homocysteine. The body mass index was also positively correlated with homocysteine.

In the normal pregnant women, there was a negative but non-significant correlation between homocysteine and estimated foetal weight, infant birthweight, diastolic blood pressure, folate and vitamin B_{12}. Homocysteine showed positive correlations with all the lipid parameters (total cholesterol, triglycerides, LDL-C and HDL-C), with the correlation with total cholesterol, triglycerides and LDL-C being statistically significant.

Table 4.7: Pearson's Correlation Co-efficient for Demographic, Clinical, and Biochemical Parameters in PIH (Lower Left-Hand Side) and NP (Upper Right-Hand Side)

PMT	Age	GA	BMI	SBP	DBP	EFW	BWT	TC	TG	LDL	HDL	HCY	B12	Folate
Age		-0.198	0.199	0.028	-0.111	-0.302	0.155	0.348	0.226	0.458*	0.174	0.218	-0.086	0.176
GA	-0.120		0.014	0.283	0.446*	0.310	0.025	-0.453*	-0.336	-0.272	-0.423*	0.042	0.008	-0.070
BMI	-0.010	-0.147		0.229	0.255	-0.265	-0.030	0.236	0.428*	0.283	0.150	0.294	0.104	-0.430*
SBP	0.229	-0.244	0.114		0.655**	0.043	-0.113	-0.247	-0.077	-0.148	-0.241	0.116	-0.193	-0.053
DBP	0.065	-0.187	0.157	0.834**		-0.026	-0.011	-0.435*	-0.096	-0.361*	-0.308	-0.126	0.154	-0.125
EFW	-0.152	0.407**	-0.163	-0.256*	-0.142		0.083	-0.168	-0.208	-0.167	-0.280	0.137	-0.082	-0.104
BWT	-0.204	0.410**	-0.112	-.142	-0.131	0.736**		-0.354	-0.134	-0.272	-0.245	-0.276	-0.194	0.281
TC	-0.011	0.038	0.111	0.044	0.143	0.230	-0.006		0.581**	0.895**	0.608**	0.442*	0.097	-0.202
TRG	0.158	0.074	0.052	0.429**	0.346**	-0.104	-0.095	0.185		0.537**	0.261	0.414*	-0.021	-0.246
LDL	-0.110	-0.160	0.036	0.142	0.258*	0.091	-0.091	0.846**	0.140		0.441*	0.448**	-0.090	-0.109
HDL	0.191	0.373**	0.140	-0.310*	-0.282*	0.271*	0.209	0.247	0.120	-0.215		0.286	0.068	-0.283
HCY	0.059	-0.300*	0.163	0.334**	0.389**	-0.568**	-0.374**	0.002	0.254*	0.093	-0.282*		-0.050	-0.237
B12	-0.104	0.108	0.088	0.034	0.019	0.253	0.231	0.055	-0.015	0.051	-0.042	-0.174		-0.064
Folate	-0.059	-0.051	0.092	0.055	-0.142	0.268	0.308*	0.001	-0.239	-0.047	0.110	-0.283*	0.170	

Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed). PIH: Pregnancy Induced Hypertension; NP: Normal Pregnant women; PMT: parameter; GA: Gestational Age; BMI: Body Mass Index; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; EFW: Estimated Foetal Weight; BWT: Birth Weight; TC: Total Serum Cholesterol; TRG: Triglycerides; LDL: Low Density Lipoprotein Cholesterol; HDL: High Density Lipoprotein Cholesterol; HCY: Serum Homocysteine; B_{12}: Serum Vitamin B_{12}.

4.8 PEARSON CORRELATION BETWEEN THE STUDIED CLINICAL VARIABLES IN PREECLAMPSIA AND GESTATIONAL HYPERTENSION.

Table 4.8 shows the correlation between the studied parameters in the preeclamptic and the gestational hypertensive patients.

In the PE patients, even though homocysteine showed negative correlation with estimated foetal weight, infant birthweight, folate, gestational age and vitamin B_{12}, these correlations were not statistically significant at $P<0.05$. Systolic blood pressure and diastolic blood pressure are positively correlated with homocysteine. However, only diastolic blood pressure showed a statistically significant positive correlation with homocysteine. The lipid parameters (total cholesterol, triglycerides, LDL-C and HDL-C TC) showed a positive but non-significant correlation with homocysteine. The body mass index was also positively correlated with homocysteine.

In the GH patients, although homocysteine showed negative correlation with estimated foetal weight, infant birthweight, total cholesterol, LDL-C, body mass index, gestational age, folate and vitamin B_{12}, it was only the correlations between estimated foetal weight, total cholesterol and LDL-C that were statiscally significant. Systolic blood pressure, diastolic blood pressure and triglycerides showed positive correlation with homocysteine, with the correlation between diastolic blood pressure and homocysteine statistically significant.

Table 4.8: Pearson's Correlation Co-efficient for Demographic, Clinical, and Biochemical Parameters in PE (Lower Left-Hand Side) and GH (Upper Right-Hand Side)

PMT	Age	GA	BMI	SBP	DBP	EFW	BWT	TC	TG	LDL	HDL	HCY	B12	Folate
Age		0.179	-0.251	0.274	0.208	0.175	0.115	0.243	0.203	0.165	0.176	-0.067	-0.124	0.044
GA	-0.244		-0.092	0.068	0.162	0.404*	0.271	-0.029	0.014	-0.234	0.296	-0.110	0.170	-0.267
BMI	0.112	-0.151		0.020	0.000	0.107	0.153	0.129	0.073	0.116	0.002	-0.106	0.256	0.082
SBP	0.191	-0.279	0.142		0.778**	-0.220	0.055	-0.001	0.438*	0.108	-0.392*	0.243	0.105	-0.181
DBP	-0.022	-0.271	0.216	0.858**		-0.079	0.001	-0.175	0.432*	0.113	-0.458**	0.385*	-0.005	-0.374*
EFW	-0.403*	0.146	-0.350	-0.096	-0.063		0.600**	0.259	-0.223	-0.009	0.335	-0.391*	0.243	0.280
BWT	-0.377*	0.425*	-0.139	-0.255	-0.366*	0.433*		-0.013	-0.228	-0.076	0.084	-0.313	0.208	0.304
TC	-0.200	0.038	0.111	0.108	0.312	0.209	-0.127		0.094	0.795**	0.367*	-0.489**	0.162	0.214
TRG	0.121	.152	0.036	0.457*	0.306	0.018	-0.039	0.397*		0.042	0.152	0.477**	-0.019	-0.225
LDL	-0.303	-0.108	-0.019	0.153	0.343	0.292	-0.078	0.908**	0.243		-0.117	-0.332*	0.086	0.165
HDL	0.287	0.276	0.319	-0.144	-0.107	-0.244	-0.137	0.093	0.120	-0.311		-0.258	0.060	0.062
HCY	0.042	-0.012	0.179	0.256	0.441*	-0.180	-0.258	0.326	0.358	0.248	0.087		-0167	-0.348
B12	-0.071	-0.040	-0.033	0.053	0.093	0.144	-0.140	-0.114	-0.001	0.029	-0.368*	-0.062		0.269
Folate	-0.165	-0.114	0.178	0.242	0.166	-0.112	0.082	-0.414*	-0.288	-0.348	-0.079	-0.026	-0.178	

Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed). PE: Preeclampsia; GH: Gestational Hypertension; PMT: parameter; GA: Gestational Age; BMI: Body Mass Index; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; EFW: Estimated Foetal Weight; BWT: Birth Weight; TC: Total Serum Cholesterol; TRG: Triglycerides; LDL: Low Density Lipoprotein Cholesterol; HDL: High Density Lipoprotein Cholesterol; HCY: Serum Homocysteine; B_{12}: Serum Vitamin B_{12}.

Chapter 5

DISCUSSION

Hypertensive disorders of pregnancy are a common complication occurring during pregnancy which are associated with maternal and fetal morbidity and mortality. In spite of the advances in research, it is still stands as a public health problem. (Parmar *et al.*, 2012). The homocysteinemediated vascular changes are similar to those associated with pregnancy induced hypertension (PIH); therefore a hypothesis has been proposed that elevated homocysteine may be associated with this condition (Mahal *et al.*, 2009). An elevated concentration of total circulating homocysteine in serum is recognized as an important and independent risk factor for cardiovascular diseases. Moreover, the determinants of hyperhomocysteinaemia, such as low concentrations of folic acid and vitamin B_{12} involved in homocysteine metabolism are also associated with increased risk of vascular damage (Mahal *et al.*, 2009; Mujawar *et al.*, 2011).

This case-control study evaluated the plasma homocysteine, vitamin B_{12} and folate levels among Ghanaian with normal pregnancy and pregnant women presenting with pregnancy induced hypertension (PIH), and how these parameters affect pregnancy outcome. It also identified factors that may contribute to an increased risk of PIH, preeclampsia (PE) and gestational hypertension (GH). The findings indicated that the homocysteine levels were significantly elevated in the preeclamptic and gestational hypertensive women on comparison with normal pregnant controls.

5.1 HOMOCYSTEINE, VITAMIN B_{12} AND FOLATE LEVELS IN PIH

The findings of this study suggests that levels of serum homocysteine, folic acid and vitamin B_{12} are significantly altered in PIH, PE and GH patients when compared with age-matched normotensive pregnant control subjects. The presence of significant hyperhomocysteinaemia in

PE is consistent with the findings of other studies (Blad *et al.*, 2005; Khosrowbeygi & Ahmadvand, 2011; Mujawar *et al.,* 2011; Mukhopadhyay *et al.,* 2014). However, this finding contradicts the finding of Zeeman *et al.* (2003) where the authors showed that there was no significant difference in maternal serum levels of homocysteine between preeclampsia and normal pregnancy. The presence of significantly low levels of vitamins B_{12} and folate in this study in the preeclamptic women compared to the normal pregnant women suggests that there were deficiencies of vitamin B_{12} and folate. This finding is consistent with the findings of Mujawar *et al.* (2011) and Mignini *et al.* (2005). Conversely, some studies found no significant difference in folic acid and vitamin B_{12} levels between preeclamptic pregnant women and normal pregnant women (Braekke *et al.*, 2007; Guven *et al.*, 2009; Lachmeijer *et al.*, 2001; Makedos *et al.*, 2007). López-Quesada *et al.* (2003) found that the levels of homocysteine and folate are increased in preeclampsia, but values of vitamin B_{12} are not changed compared with normal pregnancy.

This study also found that the serum homocysteine concentration is inversely (negatively) correlated with serum vitamin B_{12} and folate in PIH patients, PE patients and GH patient. However, in the PIH patients, there was a statistically significant negative correlation between homocysteine and folate ($r = -0.283$, $P<0.05$). The presence of negative correlation between homocysteine and B vitamins (vitamin B_{12} and folate) levels is consistent with the findings of other studies (Guerra-Shinohara *et al.*, 2002; Mujawar *et al.*, 2011).

In uncomplicated normal pregnancies, maternal circulating level of homocysteine is decreased (Holmes *et al.*, 2005; Ingec *et al.*, 2005; Murphy *et al.*, 2002). Several explanations have been proposed for the lower total homocysteine concentrations in pregnancy, including hemodilution (the increase in gestational blood volume begins at 6 to 10 weeks, proceeds rapidly during the second trimester, and peaks at 30 to 34 weeks- the average total increase is 1.2 to 1.5 L), increased

glomerular filtration rate, increase in oestrogen level, decreased albumin concentrations during pregnancy, or increased demand for methionine by both mother and foetus (Ingec *et al.*, 2005; Patel *et al.*, 2012), and increases in enzymatic activity related to homocysteine metabolism (Dasarathy *et al.*, 2010).

Several factors may increase homocysteine levels in women with pregnancy induced hypertension. The hyperhomocysteinaemia found in the PIH (PE and GH) patients might be due to modulation in homocysteine metabolism (Mujawar *et al.*, 2011). The kidney is one of the major route through which homocysteine is cleared from plasma and this routes of elimination may be affected by preeclamptic changes in the kidney (Bostom & Lathrop, 1997; Mujawar *et al.*, 2011).

According to Guerra-Shinohara *et al.* (2002), the presence of low serum folate values in the pregnant women is an expected outcome, considering that vitamin B_{12} deficiency can interfere with folate metabolism. In the absence or deficiency of vitamin B_{12}, folate is 'trapped' and cannot be recycled back into the folate pool, hence leading to decreased folate levels (GuerraShinohara *et al.*, 2002). Allen (1997) does not believe that pregnant women have vitamin B_{12} deficiency since a normal diet would satisfy their daily requirements. The maternal organism has a store of about 5000 µg of this vitamin, mainly in the liver, which may be used under the overload conditions imposed by pregnancy (Allen, 1997). However, in an under-developed country like Ghana, where chronic nutritional deficiency exists side by side with poverty, conditions such as families with large numbers of individuals, unemployment and low per capita income may prevent the more underprivileged population from obtaining a diversified diet.

Vitamins B_{12} and folate play central roles in homocysteine metabolism. Current literature reveals that vitamin B_{12} and folate are required for the metabolism of homocysteine, through either remethylation or transsulfuration (Mujawar *et al.*, 2011). If vitamin B_{12} and folate are not present

in adequate amounts to support these metabolic changes, then the natural decrease of homocysteine might not occur and hyperhomocysteinaemia may develop. Vitamin B_{12} and folate deficiencies might thus confer a greater risk of pregnancy induced hypertension on the mother (Makedos *et al.*, 2007). One way of confirming inadequate cellular folate and vitamin B_{12} concentrations is by determining serum total homocysteine levels, which have been considered to be a sensitive functional marker of these deficiencies (Guerra-Shinohara *et al.*, 2002; Scholl & Johnson, 2000). As such, the significantly high homocysteine levels observed in the PIH, PE and GH patients in comparison with the normal pregnant women could be as a result of the low levels of vitamin B_{12} and folate observed in these patients.

Although genetic mutations were not explored in this study, one of the most common genetic polymorphisms associated with mild hyperhomocysteinaemia is a point mutation in the 5,10methylenetetrahydrofolate reductase (MTHFR) gene, a C -to-T substitution at nucleotide 677 (C677T). However, this mutation has a very low incidence among black populations (Bailey & Gregory, 1999; Frosst *et al.*, 1995; Giles *et al.*, 1998; Gudnason *et al.*, 1998; Stevenson *et al.*, 1997). Furthermore, Makedos *et al.* (2007) reported that hyperhomocysteinaemia caused by the methylenetetrahydrofolate reductase C677T mutation, can be corrected with folic acid administration. As such, it is expected that the folate supplement taken by the pregnant women as part of their routine antenatal care should be able to correct the presence of methylenetetrahydrofolate reductase C677T mutation (if any) that could result in hyperhomocysteinaemia. Also, several studies have investigated the incidence of this polymorphism among women with preeclampsia (Grandone *et al.*, 1997; Lachmeijer *et al.*, 2001; Powers *et al.*, 1999; Sohda *et al.*, 1997). The majority of these studies report no significant increase in the prevalence of this polymorphism among women with preeclampsia compared with women

with a normal pregnancy outcome. As such, it can be deduced that the high homocysteine levels observed in the PIH, PE and GH patients in comparison to the normal pregnant women may not be as a result of point mutation of the MTHFR gene.

In this present study, the levels of vitamin B_6, a cofactor in the transsulfuration pathway in homocysteine metabolism, was not assessed. However, the association of low levels of vitamin B_6 and cardiovascular disease has been reported to be independent of homocysteine when studied in general populations (Rimm *et al.*, 1998; Robinson *et al.*, 1998; Verhoef *et al.*, 1996). It is, therefore, possible that the hyperhomocysteinaemia observed in the PIH, PE and GH patients may not be due to the deficiency of vitamin B_6.

Some studies have indicated that hyperhomocysteinaemia can be treated with a combination of vitamin B_{12} and folate (de Vries *et al.*, 1997; Mansour *et al.*, 2011; Refsum *et al.*, 2006). Appropriate vitamin supplementation not only improves vitamin status and lowers homocysteine concentrations (McKay *et al.*, 2000), but it is also relatively inexpensive and easy to administer. As part of the antenatal care, all of the pregnant women in this present study were being supplemented with vitamins B_{12} and folate. One might presume that the use of vitamin and mineral supplements containing folic acid and vitamin B_{12} would offset the risk of low vitamin B_{12} and folate levels, particularly because the folic acid contained in supplements has greater bioavailability than does the folate in food. However, there still exist significantly low levels of vitamin B_{12} and folate in the PIH patients. Several factors could account for the significantly low levels of vitamin B_{12} and folate, despite the supplementation. Nallamothu *et al.* (2002), however, showed that the addition of vitamin B_{12} to folic acid supplementation achieves little additive effect in lowering homocysteine. More so, this could result from the improper defined and monitored supplement regimen, coupled with the additional nutrient demands of pregnancy.

There is no evidence of adverse effects of high folate vitamin B_{12} levels on mothers during pregnancy, caused by the supplementation dose used. However, information on the lowest dose of vitamin supplementation needed to correct the hyperhomocysteinaemia is scarce (de Vries *et al.*, 1997).

The dietary pattern of the pregnant women could also account for the low folate levels. Leafy green vegetables remain one of the major food sources of folate. There was a statistically significant difference ($P<0.05$) in the dietary intake of green leafy vegetables among the PIH patients when compared with the normal pregnant women (Table 4.4). Although behavioral factors such as cigarette smoking, alcohol intake, or using oral contraceptives are also associated with poor folate status, none of the recruited pregnant women had the listed factors. There was however no significant difference in the consumption of meat and fish amongst the study participants (Table 4.4).

Consequently, with the presence of an inverse relationship between homocysteine and B vitamins (vitamin B_{12} and folate) in the pregnant women, coupled with a significant negative correlation between the homocysteine and folate levels ($r = -0.283$, $P<0.05$) in the PIH patients, it can be concluded that the significant hyperhomocysteinaemia observed in PIH, PE and GH in comparison to the control group might be as a result of the lower levels of vitamin B_{12} and folate in these groups than in the control group.

The exact mechanism by which hyperhomocysteinaemia causes endothelial cell damage is not known. Generation of hydrogen peroxides, depletion of nitric oxide-mediated detoxification of homocysteine, enhanced endothelial cell factor V activity, and impaired endothelial thrombomodulin expression are possible aetiologic factors (de Vries *et al.*, 1997).

Hyperhomocysteinaemia was known to be associated with an approximately 20 to 3-fold increased risk of pregnancy-induced hypertension (Steegers-Theunissen *et al.,* 2004). Elevated homocysteine injuries and abnormal vascular endothelium have been observed in preeclampsia, which contribute to the pathogenesis of the preeclampsia (Patel *et al.*, 2012). In addition, vascular endothelium in pregnant women may be more sensitive to homocysteine injury. Therefore moderate elevation of total homocysteine level in pregnancy induced hypertension compared to normal pregnant may play a role in endothelial injury with subsequent activation of various factors that eventually results in this condition (Patel *et al.,* 2012).

5.2 HYPERHOMOCYSTEINAEMIA AND PREGNANCY OUTCOMES

Severe pregnancy outcomes such as intrauterine growth restriction (IUGR) and low birthweight (LBW) occur in spite of regular antenatal care. The causes for IUGR still remain unknown, although several determinants have been identified (Infante-Rivard *et al.*, 2003). Hyperhomocysteinemia has been reported to affect foetal development through intrauterine growth restriction and is one of the important issues associated with low birth weight of newborns (de Vries *et al.*, 1997; Steegers-Theunissen *et al.*, 2004; Vollset *et al.*, 2000).

Little is known about the relation between total homocysteine (tHcy) levels, B vitamin status and birth outcomes among Ghanaian pregnant women. This, therefore, necessitated the quest to assess the correlation between serum homocysteine levels, B vitamin status and adverse pregnancy outcomes in pregnancy induced hypertension. This present study showed that while none of the normal pregnant women had foetal growth restriction or low birthweight babies, 35.0% and 28.3% of the participants presenting with pregnancy induced hypertension had intrauterine growth restriction and low birthweight, respectively (Table 4.5) Except for the GH patients where estimated foetal weight (EFW) was insignificantly lower, EFW and infant birthweight were

significantly lower in the PIH (PE and GH) patients in comparison with the normal pregnant women (Table 4.2). Similar to this study, significantly lower estimated foetal weight (EFW) were observed in preeclamptic patients (Bergen *et al.*, 2012; Laskowska & Oleszczuk, 2011; Lindblad *et al.*, 2005). Contrary to the findings of this present study, InfanteRivard *et al.* (2003) concluded that the probability of a mother giving birth to a baby with growth restriction decreased with increasing tHcy; and birthweight increased with increasing tHcy concentration. The observed lower birth weights in babies of mothers with the highest tHcy agrees with some other studies (de Vries *et al.*, 1997; Vollset *et al.*, 2000), but contradicts the findings of Infante-Rivard *et al.* (2003) and Ronnenberg *et al.* (2002). This study also showed a statistically significant negative correlation between EFW, infant birthweight and homocysteine concentration in PIH patients. The findings of this study, therefore, suggest that tHcy plays an important role in the aetiology of adverse pregnancy outcomes. This study demonstrated that EFW and infant birthweight correlated positively with folate and vitamin B_{12} in the PIH patients. However, infant birthweight correlated significantly with folate levels in the PIH patients.

Wang *et al.* (2000) in their study concluded that elevated plasma homocysteine plays a role in the pathogenesis of the vascular disease in the uteroplacental circulation in placental insufficiency, and these results were consistent with the hypothesis for the vascular lesion in maternal uteroplacental bed in both preeclampsia and fetal growth restriction. It has been speculated that elevated homocysteine may also compromise pregnancy outcomes by interfering with connective tissue integrity (Ferguson *et al.*, 2001). Vitamin B_{12} and folate are critically important for fetal development. Vitamin B_{12} is critical for nucleotide synthesis and amino acid metabolism. Once absorbed, folate acts as a cofactor for many essential cellular reactions including the transfer of single-carbon units; it is required for cell division because of its role in DNA synthesis (Scholl &

Johnson, 2000). The role of folate in DNA synthesis and cell replication suggests that folate can influence fetal growth and gestation duration. Folate deficiency also interferes with growth of the conceptus, maternal erythropoiesis, growth of the uterus and mammary gland, and growth of the placenta. Therefore, it appears plausible that deficiencies of this vitamins could cause hyperhomocysteinaemia which contributes to chorionic, hormonal, or other abnormalities involved in IUGR and LBW (Scholl & Johnson, 2000). Some previous studies have suggested that maternal B vitamin status may influence the risk of both IUGR and LBW. Hibbard (1975) reported an association between maternal red blood cell folate concentrations at or before 16 weeks of gestation and the proportions of small-for-gestationalage infants. In another study, Scholl *et al.* (1996) found that lower dietary intake of folate and lower serum folate at week 28 were associated with a 3-fold increase in the risk of LBW. Because foetal growth is greatest in the last trimester of pregnancy (Ronnenberg *et al.*, 2002), the deficiency in the availability of folate and vitamin B_{12} will ultimately affect pregnancy outcome.

Regardless of whether the associations between homocysteine, B vitamin status, LBW and IUGR are independent, numerous intervention trials have shown that appropriate vitamin supplementation improves B vitamin status and lowers homocysteine concentrations (McKay *et al.*, 2000). Vitamin supplementation trials among pregnant women are needed in this population to determine whether improving vitamin nutritional status also reduces the risk of IUGR and LBW. It may be concluded from this study that maternal hyperhomocysteinaemia during pregnancy is a risk factor for IUGR and small size newborns in our population. Thus, antenatal checkup of pregnant mothers for hyperhomocysteinaemia appears to be important.

5.3 LIPID PROFILE IN PREGNANCY INDUCED HYPERTENSION AND ITS CORRELATION WITH HOMOCYSTEINE, VITAMIN B_{12}, FOLATE

Human gestation is associated with an atherogenic lipid profile that is further enhanced in PIH (PE and GH). Dyslipidemia is believed to be a potential contributor to endothelial cell dysfunction, which is a central feature in the pathophysiology of hypertensive pregnancies (Mahal *et al.*, 2009). The endothelial dysfunction in preeclampsia could originate from oxidative stress as well as dyslipidaemia (Cekmen *et al.*, 2003).

This present study demonstrated that lipid profiles are altered in hypertensive pregnancies (Table 4.2). The significantly low serum HDL-C concentration observed in the PIH and PE patients when compared to the normal pregnant women is similar to the findings of other studies (Belo *et al.*, 2002; Gohil *et al.*, 2011; Mahal *et al.*, 2009; Malli *et al.*, 2013; Saxena *et al.*, 2015). The study by Ephraim *et al.* (2014) and Ahenkorah *et al.* (2008) indicated that though the mean HDL-C was higher in controls than in PIH patients, the differences were of no statistical significance. Evrüke *et al.* (2004) and Demir *et al.* (2011), however, observed no difference in the HDL-C between the PIH patients and normal pregnant women, while Bai *et al.* (2002) observed higher HDL-C level in PIH patients. The significantly low HDL-C observed in the PIH and PE patients might be as a result of the elevated triglycerides observed in this study. Elevated triglycerides play a part to decrease the maternal HDL-C level (Saxena *et al.*, 2015). A direct correlation between adipose tissue lipoprotein lipase activity and plasma HDL-C has been established which may be responsible for low levels of HDL-C. Decreased HDL-C resulting from hypertriglyceridaemia, is mainly due to the action of cholesteryl ester transfer protein (Saxena *et al.*, 2015).

Similar to the findings of this study, other researchers (Ahenkorah *et al.*, 2008; Ephraim *et al.*, 2014; Gohil *et al.*, 2011; Mahal *et al.*, 2009; Malli *et al.*, 2013; Saxena *et al.*, 2015) observed higher concentrations of triglycerides in PIH (PE and GH) patients in comparison to normal

pregnant women. In contrast, Demir *et al.* (2011) observed no significant difference in the triglycerides levels. The principal modulator of hypertriglyceridaemia is oestrogen since pregnancy is associated with hyperoestrogenaemia. Oestrogen induces hepatic biosynthesis of endogenous triglycerides, by increasing the hepatic very low density lipoprotein-cholesterol (VLDL-C) synthesis (Irinyenikan *et al.*, 2014; Saxena *et al.*, 2015). The activities of adipose tissue lipoprotein lipase and hepatic lipase are substantially decreased during pregnancy due to insulin resistance and oestrogen respectively (Saxena *et al.*, 2015). Moreover, the increased, but not significant BMI observed in the PIH (PE and GH) patients could partly explain the significant increase in triglycerides and LDL because increase in weight and BMI is associated with increase in body fat percentage levels (Sanlier & Yabanci, 2007). Although it is still unclear whether hypertriglyceridemia becomes a risk factor for preeclampsia or whether there is any causal association between them, high triglyceride levels seem to increase the risk of placental vascular disorders, which trigger endothelial dysfunction (Lima *et al.*, 2011). It has therefore been hypothesized that the increased triglyceride found in PIH may be deposited in predisposed vessels, such as the uterine spiral arteries and consequently contributes to the endothelial dysfunction both directly and indirectly through generation of small, dense low density lipoprotein cholesterol (Irinyenikan *et al.*, 2014; Saxena *et al.*, 2015). The development of atherosclerosis in the placental spiral arteries of preeclamptic women is, therefore, an indication that elevated levels of triglycerides are involved in PIH (Al-Jameil *et al.*, 2014). Moreover, this hypertriglyceridemia may be associated with hypercoagulability (De *et al.*, 2006; Lima *et al.*, 2011).

Although the levels of total cholesterol were higher in the PIH (PE and GH) patients than in the normal pregnant women, this elevation was insignificant. This is in keeping with the study of Ahenkorah *et al.* (2008), Irinyenikan *et al.* (2014) and De *et al.* (2006). However, contrary to the

findings of this study, the study by Ephraim *et al.* (2014) showed that total cholesterol levels were significantly elevated in PE patients in comparison with the control.

This present study also observed significantly ($P<0.05$) high levels of LDL-C in the PIH and PE patients than in the control group, which is consistent with the findings Ephraim *et al.* (2014), Ahenkorah *et al.* (2008), Gohil *et al.* (2011), Mahal *et al.* (2009) and Saxena *et al.* (2015) but contradicts the findings of Demir *et al.* (2011) and Kaloti *et al.* (2013) where no significant relationship was observed. Abnormal concentrations of plasma lipoprotein is a major modifiable risk factor for cardiovascular diseases. An increase in LDL-C predisposes the pregnant women to the development of atherosclerosis. Similarly, low HDL-C levels also predisposes one to atherosclerosis (Al-Jameil *et al.*, 2014; Irinyenikan *et al.*, 2014).

Women with preeclampsia are known to develop arterial lesions at the utero-placental implantation site. These morphological lesions, which are characterized by areas with fibrinoid necrosis surrounded by lipid-laden macrophages, are usually observed in cases of acute atherosclerosis. Furthermore, glomerular endotheliosis, the presence of lipid deposits in the glomeruli, is observed in preeclamptic patients. Glomerular lesions are associated with proteinuria, a predictive indicator and marker of disease severity. It has also been suggested that LDL-C and triglycerides may be involved in this renal damage (Al-Jameil *et al.*, 2014). Our findings suggest that lipids may be involved in the endothelial damage observed in hypertensive disorders of pregnancy.

Although the pattern of hyperlipidaemia (high total cholesterol, triglycerides and LDL-C) in the GH patients are similar to that of the PE patients in comparison with the normal pregnant women, the differences in the lipid fractions of the GH patients and normal pregnant women are insignificant. These values were higher in the PE patients than in the GH patients. This is in keeping with the findings of Theresa *et al.* (2013). This variation suggests that dyslipidaemia in

pregnancy has a significant effect on preeclampsia than gestational hypertension. This could further complicate the condition of PE patients.

The variation in the findings of dyslipidaemia in our participants when compared with other studies might be explained, in part, by differences in research design/ methodology, small sample sizes, nutrition or possibly due to the differences in study populations. Another point of concern is with the sampling time. While the available body of literature is vast, it is hard to make direct comparisons between studies as the gestational age at which the lipid measurements were taken vary widely from preconception to postpartum. Also, the fact that 50% of the PIH patients used contraceptives prior to their pregnancy could account for this variation in the lipid profile. However, it has been observed that the lipid profile findings of this study are similar to the other studies conducted in other parts of Ghana (Ahenkorah *et al.*, 2008; Ephraim *et al.*, 2014).

With such high levels of inconsistency, it is difficult to conclude, with any level of certainty, the relationship between lipid levels during pregnancy and preeclampsia risk.

In this study, homocysteine levels showed a significant negative correlation with HDL-C and a significant positive correlation with triglycerides in the PIH patients. Similar to the study of Mahalle *et al.* (2014) and Xiao *et al.* (2011), we observed that serum homocysteine was negatively correlated with HDL-C level in cardiovascular diseases.

The positive correlation between homocysteine and triglycerides observed in this study is consistent with the findings of Mahalle *et al.* (2014). However, other population based studies on cardiovascular diseases reported no correlation between Hcy and lipids (Shai *et al.*, 2004; Yadav *et al.*, 2006). Furthermore, some animal studies have also confirmed the negative correlation between HDL-C with homocysteine in mice (Mahalle *et al.*, 2014; Mikael *et al.*, 2006).

Homocysteine, like other sulfhydryl compounds, may promote the oxidation of LDL cholesterol, reduce the concentration of HDL cholesterol in plasma by inhibiting the synthesis of Apo A-I, the main HDL apolipoprotein and increase the serum levels of MDA. Homocysteine induced lipid dysregulation is an important mechanism linking Hcy to the development of atherosclerosis (Saleh, 2015). The strong relationship between serum homocysteine levels and HDL-C concentrations observed in this study may increase cardiovascular risk. Moreover, further studies with a larger sample size are, however, required to determine the role of the lipid status with respect to serum homocysteine levels in women presenting with pregnancy induced hypertension.

Though homocysteine is known to be an independent risk factor for developing cardiovascular disease, the significant correlation between decreased HDL-C and elevated homocysteine level in pregnancy induced hypertension implies that both may have additive or synergistic effects on the pathophysiology of pregnancy induced hypertension.

5.4 RELATIONSHIP BETWEEN BLOOD PRESSURE (SBP, DBP), BMI AND HOMOCYSTEINE.

High blood pressure (elevated systolic blood pressure [SBP] and diastolic blood pressure [DBP]) is a major and an independent risk factor for cardiovascular disease (Animesh & Mehrotra, 2014; Atif *et al.*, 2008; van Guldener *et al.*, 2003). However, its aetiology has not been fully elucidated mostly because of unknown genetic variation. Furthermore, multiple non-hereditary factors including dietary and other lifestyle factors have been identified that have important and modifiable influences on blood pressure (Animesh & Mehrotra, 2014). Although controversy remains as to whether the relation between homocysteine and cardiovascular disease is causal or

not, some studies have suggested that mild elevations in serum homocysteine may contribute to elevations in blood pressure (Animesh & Mehrotra, 2014; Lim & Cassano, 2002; van Guldener *et al.*, 2003).

In this study, there was a significant ($P<0.01$) positive correlation between homocysteine and blood pressure (SBP, DBP) in the PIH patients. In the PE and GH patients however, it was only the DBP that showed significant ($P<0.05$) correlation. Some previous studies did not find any association between homocysteine and blood pressure, and some did find a significant, although weak, association between plasma homocysteine and blood pressure (van Guldener *et al.*, 2003). The positive association of blood pressure (SBP, DBP) with homocysteine is consistent with the findings of other studies (Lim & Cassano, 2002). Unlike the review of van Guldener *et al.* (2003) where the authors concluded that high blood pressure (especially systolic hypertension) has been linked to elevated plasma homocysteine in several studies, this study observed significant association between DBP and homocysteine in the PE and GH patients. It is unclear why the findings of this study differ from these earlier trials. However, the differences in the sample sizes used might be a factor. Nevertheless, the PIH patients showed significant ($P<0.05$) association between blood pressure (SBP, DBP) and homocysteine. It has been demonstrated that homocysteine-lowering treatment is associated with a reduction in systolic and diastolic blood pressures, suggesting a potential causal role for homocysteine in the pathogenesis of elevated blood pressure. Thus, a considerable body of evidence suggests a role for plasma homocysteine in the pathogenesis of hypertension (Sundström *et al.*, 2003). The presence of a significant positive correlation between blood pressure and homocysteine, therefore, supports the argument that homocysteine plays a pivotal role in the pathogenesis of pregnancy induced hypertension.

Homocysteine may elevate blood pressure by causing arterial stiffness due to impaired vascular endothelial integrity and/or by reducing the efficiency of vasodilation. Furthermore, reduced

methylation potential due to elevated homocysteine and S-adenosyl-homocysteine may compromise the growth and integrity of the endothelial cells, hence leading to endothelial dysfunction. In addition, homocysteine generates free radicals during its auto-oxidation to thiolactone and inhibits the expression of antioxidant enzymes, such as glutathione peroxidase (Lim & Cassano, 2002; Sundström *et al.*, 2003; van Guldener *et al.*, 2003).

Many studies have shown that elevated tHcy and obesity are both associated with increased cardiovascular disease risk (Yilmaz *et al.*, 2014). However it is unclear if these two risk factors are interrelated. The excess weight in pregnancy has been reported to be associated with preeclampsia and other hypertensive disorders of pregnancy and later on in life with cardiovascular disease (Bautista-Castaño *et al.*, 2013; Sohlberg *et al.*, 2012).

Although the BMI in the PIH (PE and GH) patients in this study were higher than that of the normal pregnant women, it was only in the PIH patients that there was statistically significant difference ($P<0.05$). This study showed that the participants with PIH (PE and GH) were more obese than the normal pregnant women, and this is in agreement with other studies (Sohlberg *et al.*, 2012; Vinod *et al.*, 2015). A meta-analysis of maternal BMI and preeclampsia showed that the risk was doubled with every 5 to 7 unit increase in pre-pregnancy BMI (Bautista-Castaño *et al.*, 2013; Vinod *et al.*, 2015). Furthermore, some authors reported that the risk of preeclampsia during pregnancy doubled in overweight women (25-29.9 kg/m^2), while it was 4.5 times higher in obese women (30-39.9 kg/m^2) (Vinod *et al.*, 2015).

Furthermore, the insignificant correlation between homocysteine and BMI observed in this study is in keeping with the findings of other studies (Brasileiro *et al.*, 2005; Yilmaz *et al.*, 2014). Even though the pathophysiological mechanism by which increased BMI predisposes women to developing pregnancy induced hypertension is not fully understood, increased insulin resistance is

likely an important contributing factor (Ephraim *et al.*, 2014; Owiredu *et al.*, 2012). Insulin resistance has been demonstrated to be associated with elevated plasma homocysteine levels in non-obese subjects. Gallistl *et al.* (2000) in their study to determine the correlation between plasma homocysteine levels in obese children and adolescents observed that in obese children and adolescents, tHcy levels were strongly related with body mass index and insulin, suggesting that hyperinsulinism associated to obesity may contribute to impairment of homocysteine metabolism. The mechanism by which insulin, or possibly one of its counter-regulatory hormones, regulates the enzyme activities of the hepatic transsulfuration pathway is unknown (Jacobs *et al.*, 1998).

However, since significant differences in BMI between the cases and the normal pregnant women occurred only in the PIH patients, and not in the PE patients where the highest mean homocysteine concentration was observed (Table 4.2), the elevated homocysteine concentration in the PIH (PE and GH) patients on comparison with the control group might not result from insulin resistance. Consequently, it can be concluded that obesity did not influence the elevated tHcy levels, which were more associated with deficiency of vitamin B_{12} and folate. This further emphasize that elevated homocysteine level is an independent risk factor for developing cardiovascular disease.

5.5 CONTRACEPTIVE USE AND THE RISK OF DEVELOPING PIH

Contraceptive use by women has been associated with the risk of developing PIH. In this study, 50.0% of the PIH (PE and GH) patients used contraceptives (Depo-Provera and Norplant). Of those who once used contraceptives, 96.6% used medroxyprogesterone acetate (Depo-Provera). Contraceptive use predisposes women to risk of developing PIH (Table 4.6). This finding is in keeping with the finding of (Ahenkorah, 2009). Of significant notice is the observation that 80.0% of the preeclamptic patients have at some point in their life used the hormonal contraceptive Depo-Provera. Multivariate logistic regression showed that the use of the contraceptive, Depo-Provera,

by the women was significantly associated with about thirty-fold increase in the odds of developing preeclampsia (OR = 29.71, p<0.001).

Studies that evaluated the relationship between the use of Depo-Provera and the risk of developing PIH remain scarce. However, some researchers have looked at the relationship between Depo-Provera use and the risk of developing cardiovascular diseases (Meendering *et al.,* 2008). The use of hormonal contraceptives has become ubiquitous. Depo-Provera is used as an injectable progestin-only contraceptive. It is a popular contraceptive choice, particularly for younger premenopausal women because of the ease of use and high compliance. Ultimately,

Depo-Provera is a potent contraceptive with consequences (Dudar, 2010; Meendering *et al.*, 2008). Oestrogens is known to have a cardio-protective influence in women by improving endothelial function and low-density lipoprotein (LDL) concentrations while decreasing concentrations of endothelin-1 (ET-1) and homocysteine. In contrast, medroxyprogesterone acetate (Depo-Provera) has been shown to counteract the beneficial effects of oestrogens in animals and among some studies in postmenopausal women (Meendering *et al.*, 2008). Progesterone only contraceptives such as Depo-Provera create a hypoestrogenic effect, hence diminishing the estrogen's protective role on blood vessels (Dudar, 2010).

In a study where Lizarelli *et al.* (2009) evaluated Depo-Provera damage to blood vessels via flow-mediated dilation [(FMD) - FMD measures endothelial function], their data showed significant differences in FMD among Depo-Provera users and non-hormonal contraceptive users as well as combined oral contraceptive (COC) users and non-hormonal contraceptive users. Between the two hormonal contraceptive groups, there was no significant difference in FMD. Their data showed that Depo-Provera users also had lower total cholesterol as well as lower

LDL, where as non-hormonal contraceptive users had a higher high density lipoprotein.

Furthermore, the findings of (Meendering *et al.* (2008)) support previous literature that suggests that oral progesterone (which may be considered to have properties similar to Depo-Provera) has androgenic effects that are harmful to the cardiovascular system.

Chapter 6

CONCLUSION AND RECOMMENDATION

6.1 CONCLUSION

The findings of this study shows that homocysteine concentration is altered in pregnancy induced hypertension (preeclampsia and gestational hypertension) when compared with normal pregnant women. This imbalance appear to be more severe in the preeclamptic patients than in the gestational hypertensive patients, and it is depicted by an elevated serum concentration of homocysteine and a decreased serum concentrations of vitamin B_{12} and folate. Low levels of vitamin B_{12} and folate concentrations are probable causes of the hyperhomocysteinaemia observed in pregnancy induced hypertension (preeclampsia and gestational hypertension). Serum homocysteine showed an inverse (negative) relationship with serum vitamin B_{12} and folate in pregnancy induced hypertensive patients, with a statistically significant negative correlation between homocysteine and folate concentrations. It can then be concluded that elevated homocysteine may play a role in the pathogenesis of pregnancy induced hypertension and its progression.

The findings of this study demonstrate the presence of a strong relationship between elevated serum homocysteine levels and birth outcomes (intrauterine growth restriction and low birthweight). This suggests that hyperhomocysteinaemia in pregnancy plays a significant role in the aetiopathogenesis of intrauterine growth restriction and low birthweight.

Furthermore, a significant positive correlation was observed between blood pressure (systolic and diastolic blood pressure), suggesting that homocysteine plays a pivotal role in the pathogenesis of hypertension.

Finally, the use of the contraceptive Depo-Provera by women prior to pregnancy predisposes them to a high risk of developing pregnancy induced hypertension.

6.2 LIMITATION

1. In this study, the biomarkers were assessed during the course of the pregnancy. A prospective cohort study where these biomarkers are assessed post-partum and compared with the levels assessed during pregnancy would have given a clearer picture.

2. A detailed nutritional status of the participants were not assessed.

3. The small sample size used is a limitation of this study.

6.3 RECOMMENDATIONS

1. The measurement of serum homocysteine, vitamin B_{12} and folate early in pregnancy is necessary so as to reduce the possibility of developing pregnancy complications and outcomes such as pregnancy induced hypertension (preeclampsia and gestational hypertension), intrauterine growth restriction and low birthweight.

2. Defined and monitored vitamin B_{12} and folate supplements should be incorporated into the routine antenatal care of all pregnant women. This will prevent the risk of developing hyperhomocysteinaemia and subsequently pregnancy induced hypertension (PE/GH).

3. Women who decide to use a Depo-Provera as a contraceptive should be well educated on the benefits and harmful effects of this contraceptive.

4. This study showed that pregnant women who used Depo-Provera prior to pregnancy are at increased risk of developing preeclampsia. However, studies on the relationship between the use of this contraceptive and the risk of developing preeclampsia are scarce. Other studies, especially in Africa are needed to determine this relationship.

5. It had been suggested that homocysteine induced HDL-C and Apo A-I inhibition represent a novel mechanism by which homocysteine induces atherosclerotic cardiovascular diseases. Such conclusions were however made from animal studies. Human studies are, therefore, needed to determine the mechanism of homocysteine induced HDL-C and Apo A-I inhibition.

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APPENDIX

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

FACULTY OF HEALTH SCIENCES

SCHOOL OF MEDICAL SCIENCES

DEPARTMENT OF MOLECULAR MEDICINE

RESEARCH QUESTIONNAIRE

PERSONAL HISTORY

Identification number: ……. Date of visit: …/……/…….

Name: ……..... Age: ……..... years.

Residential area: ……..... H/No: …….....

Health Facility: ……... Mobile No: …….....

Marital Status: …… Educational background: ……

OBSTETRIC HISTORY

Gestational Age: ..... (Weeks). Expected Date of Delivery: …….

Parity: ……..... Gravidity: .....

Weight: .....(Kgs) Height: …….....(M)

Body Mass Index: ..... Blood pressure: .....(mmHg)

Have you been pregnant before? Yes ☐ No ☐

If yes, number of pregnancies… Miscarriages: ……, Still births ……, Induced abortion ……..

Abortion: ……, Preterm delivery ……., Live births …….., Multiple births ……

Birth weight ……

Intrauterine Growth Restriction …….

MEDICAL HISTORY

Do you have any of these?

Pre-eclampsia	☐ Yes	☐ No
Heart disease	☐ Yes	☐ No
Hypertension	☐ Yes	☐ No
Kidney disease	☐ Yes	☐ No
Liver diseases	☐ Yes	☐ No
Diabetes mellitus	☐ Yes	☐ No
Sickle cell disease	☐ Yes	☐ No
Severe anaemia	☐ Yes	☐ No
Folic acid deficiency	☐ Yes	☐ No
Vitamin B_{12} deficiency	☐ Yes	☐ No

Have you ever being diagnosed of any of the above condition before this pregnancy? ☐ Yes

☐ No

If yes which? ……..……..

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

Are you currently on any medications or injections? Yes ☐ No ☐

If yes, what are they? ……..……..….

Any other health complication? ……

Do you have any history of alcoholism? Yes ☐ No ☐

Have you currently drunk alcohol? ☐ Yes ☐ No

If yes, how long did you drink? ……

Have you ever smoked? ☐ Yes ☐ No

If yes, how long did you smoke? ……

Have you ever used any contraceptive prior to this pregnancy? ☐ Yes ☐ No

If yes which? ……..……..

FAMILY HISTORY

Do you have family history of any of the following conditions?

Pre-eclampsia ☐ Yes ☐ No

Heart disease ☐ Yes ☐ No

Hypertension ☐ Yes ☐ No

Kidney disease ☐ Yes ☐ No

Liver diseases ☐ Yes ☐ No

Diabetes mellitus ☐ Yes ☐ No

Sickle cell disease ☐ Yes ☐ No

Multiple pregnancies ☐ Yes ☐ No

Birth defects (specify) ☐ Yes ☐ No

…….

NUTRITIONAL DATA

	How often do you eat the following foods	None	Daily	Twice a week	Weekly
I.	Meat				

II.	Fish				
III	Green leafy vegetables				