An evaluation of serum leptin dynamics in Preeclampsia at the Korle-Bu Teaching Hospital

By

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JULY, 2016
DECLARATION

I, Emmanuel Abindau, do hereby declare that with the exception of cited references, this work is as a result of my own research. It is being presented to the University of Ghana for the award of a Master of philosophy degree in Physiology. This thesis has not been presented before to any other institution for examination and award of a degree.

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I thank Dr. Kwame Yeboah of the Department Of Physiology who made possible for me to start the Mphil program in the first place.
DEDICATION

This work is dedicated to Dr. Kwame Yeboah of the Department of Physiology for his selfless sacrifice.
ABSTRACT

Background: Preeclampsia is a peculiar human pregnancy condition with heightened immune response, oxidative stress, hypoxia and reduced angiogenesis. These features of preeclampsia which are influenced by leptin which causes endothelial dysfunction; the main pathology of this condition. Hypertension, which is a diagnostic marker of preeclampsia, is also associated with increased leptin level. This implicates leptin in preeclampsia pathogenesis. Data from Caucasians and Asians show higher leptin level in preeclamptic subjects and also leptin as a contributory factor to preeclampsia severity but such data is non-existent in the Ghanaian population.

Aim: To determine and correlate maternal serum leptin levels in normotensive pregnant and preeclamptic women and to relate these to the gestational age of delivery and birthweight.

Methodology: The study used a case control study design and recruited 144 consenting participants. Blood samples were drawn for hematological and biochemical analysis. Maternal serum obtained from the blood samples were used for leptin Quantification by ELISA.

Results: There was no significant difference in the leptin level between both cases and controls or the mild and severe preeclampsia cases with p>0.05. There was no correlation between leptin level and blood pressure for the controls, cases, mild preeclampsia and severe preeclampsia. The birth weight of cases was significantly higher than that of the controls.

Conclusion: Preeclampsia pathogenesis and severity in Ghanaian patients is not associated with maternal circulating leptin levels.
LIST OF ABBREVIATIONS AND ACRONYMS

ACOG…… American College of Obstetricians and Gynecologist

ALT…… Alanine aminotransferase

AST…… Aspartate aminotransferase

BMI…..Body mass index

ELISA…… Enzyme linked immunosorbent assay

HCT………. Haematocrit

HGB……. haemoglobin

HELLP…… Hemolysis, elevated liver enzymes and low platelets count

MCH…… Mean Cell Haemoglobin

MCHC…… Mean Cell Haemoglobin Concentration

MCV……….. Mean cell volume

MPV………. Mean platelet volume

PCT…… Platelet crit

PCV………. Packed cell volume

PLT…… Platelet count

RBC…… Red blood cell
RDW……… Red cell distribution width

VEGF……Vascular endothelial growth factor

VEGFR-1…… Vascular endothelial growth factor receptor 1

WBC……… White Blood Cell
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CHAPTER ONE

1. INTRODUCTION

1.1 BACKGROUND
Preeclampsia is a multisystem disorder characterized by hypertension and proteinuria and leading cause of maternal mortality/morbidity worldwide (Uzan et al. 2011). It is defined by a blood pressure $\geq 140/\geq 90$ mmHg and a proteinuria of $\geq 0.3$g/24 h after 20 weeks gestation in previously normotensive women (Vitoratos et al. 2012). It is the number one cause of preterm deliveries worldwide (Births & Sibai 2006) with other symptoms such as edema, shortness of breath, heartburn, vision problems, and impaired liver function and upper abdominal pain. Without early intervention preeclampsia may lead to a number of complications such as eclampsia (convulsion), Hemolysis, Elevated Liver enzymes, Low Platelet count (HELLP) syndrome, stroke and eventual death of the sufferer. This condition causes fetal growth restriction that results in low birth weight and other health conditions later in life. The occurrence of preeclampsia in the largest tertiary centre in Ghana is 7.03% with a prenatal mortality of 23.3 per 1000 births (Obed & Patience 2006). The worldwide death-toll of preeclampsia is 10-15% of all pregnancies (Uzan et al. 2011).

Preeclampsia is very difficult to detect early and treat and hence complications usually result. Hypertension and proteinuria, which are symptoms of preeclampsia, form the diagnostic markers. The discovery of new serum markers might be helpful in identifying subjects at increased risk of developing preeclampsia. There are many biological molecules/markers implicated in preeclampsia including the adipokines; leptin and adiponectin (Miehle et al. 2012).
Leptin is a paracrine and endocrine hormone produced by the adipocytes and the placenta (Lu et al. 2006). It controls satiety, energy homeostasis, inflammation, immune response, reproduction, angiogenesis and insulin sensitivity (Lu et al. 2006). Moreover, leptin regulates gonadotrophin releasing hormone and activates the sympathetic nervous system. An elevation of leptin levels has been observed in pregnancy due to the additional production from the placenta. The roles leptin play in the pregnancy include regulation of implantation and placental growth induces human chorionic gonadotrophin production in trophoblastic cells, mitogenesis and amino acid uptake.

Three major theories have been put forward as the causes of preeclampsia. These include; the abnormal spiral artery remodeling, the elevation in maternal systemic inflammation and the imbalance of anti-angiogenic and angiogenic factors (Ahmed & Ramma 2015). The three theories are based on processes or factors affected by leptin. It therefore means, if any of these theories is true then the leptin levels in these patients should show a significant change in relation to the normotensive participants.

Also, sudden weight gain has been identified as one of the major risk factors of preeclampsia. There is a linear correlation between body weight and leptin levels. Leptin has also been established to be involved in insulin insensitivity (Michle et al. 2012). Insulin insensitivity; the main cause of diabetes mellitus has been found as predisposing factors in preeclampsia (Craici et al. 2014). Preeclampsia is associated with increased insulin resistance (Kaaja et al. 1999). This goes to strengthen the suspicion that leptin plays a significant role in preeclampsia and therefore its level in preeclamptics should be significantly different in relation to the normotensives.
1.2 PROBLEM STATEMENT

Preeclampsia is one of the oldest maternal ailments and one of the most deadly maternal diseases. Some of the risk factors of preeclampsia such as sudden weight gain and diabetes mellitus are conditions influenced by leptin and hence leptin might be the underlining cause of preeclampsia (Miehle et al. 2012; Uzan et al. 2011). Also conditions such as intrauterine fetal growth restriction (IUGR), oxidative stress, hypoxia, maladapted immune response and endothelial dysfunction are associated with elevated leptin level. These suggest a significant difference in leptin levels between the preeclamptics and the normotensives, however, there is a disagreement in the literature on leptin levels in these two groups; some reporting higher leptin levels, others reporting no change in leptin level and some providing evidence of lower leptin level (Laml et al. 2001; Anim-Nyame et al. 2000; Martinez-Abundis et al. 2000). This has made it difficult if not impossible to relate leptin levels to preeclampsia pathophysiology, preeclampsia severity, gestational duration and birth weight.

Most of the research works on the role of leptin in preeclampsia have been conducted on Caucasians and Asians with little done on Africans in the motherland. There is therefore a data gap this study attempts to address.

1.3 JUSTIFICATION

The only remaining theory on preeclampsia pathogenesis implicates the imbalance of angiogenic and antiangiogenic factors as precipitators of preeclampsia symptoms with even animal models of the disease created by causing this imbalance in vivo (Ahmed & Ramma 2015). Vascular endothelial growth factor (VEGF) and its receptor 1 are respectively the angiogenic and antiangiogenic factors implicated in this process. Leptin is an angiogenic factor and also stimulate
the secretion of VEGF. Leptin also acts synergistically with VEGF and fibroblast growth factor (FGF) to facilitate angiogenesis (Park et al. 2001). Endothelial dysfunction, oxidative stress, hypoxia and heightened immune response which are common features in preeclampsia probably because of a decline in angiogenesis have been associated with leptin level change (Ahmed & Ramma 2015). Understanding the role or otherwise of leptin in these features in preeclampsia will form the bases for unraveling the pathogenesis of preeclampsia. This will also go a long way to fill the data void on the effect of leptin on preeclampsia in Africans.

1.5 AIM

The aim of this study is to evaluate maternal serum leptin dynamics in preeclampsia at the Korle Bu Teaching Hospital.

1.6 OBJECTIVES

- To determine serum leptin levels in normotensive pregnant women after 20 weeks gestation
- To determine serum leptin levels in preeclamptic women
- To determine and relate serum leptin levels to severity of preeclampsia and gestational age of delivery or duration of pregnancy
- To determine and relate serum leptin levels to severity of preeclampsia and birth weight.

1.4 NULL HYPOTHESIS

There is no correlation between serum leptin levels and severity of preeclampsia.
CHAPTER TWO

LITERATURE REVIEW

2.1 HISTORY OF PREECLAMPSIA/ECLAMPSIA

There is no known date that stipulates when the first preeclampsia/eclampsia was diagnosed. The knowledge of the existence of such a condition transcends the time of Hippocrates. However, Hippocrates was the first person to describe this condition in the history books around 400 BC (Preeclampsia Foundation 2013). There are also records of eclampsia in Chinese, Indian and Greek treatise some dating to about 4000 years ago (Lindheimer 2013). The recognition of eclampsia as a specific puerperal convulsion occurred in the 17th century in France during the onset of obstetrics as a discipline. Before 1739, the Greek word for eclampsia and epilepsy was the same word translated as lightening due to the sudden onset of convulsion in the two conditions (Lindheimer 2013). The French scientist, de Sauvage defined the conditions by reporting that epilepsy is a recurrent chronic condition which eclampsia is not (Bell 2011). Centuries have gone by with scores of researches carried out but the underlining cause of this condition is still a mystery to the scientific community. However, these researches have yielded some comforting outcomes with preeclampsia/eclampsia now classified out of other hypertensive disorders in pregnancy and new management methods developed.

Preeclampsia is a disorder of the vascular endothelial system that causes vasospasm, capillary leak and activation of coagulation that precipitate the symptoms of the disease. The symptoms of this condition usually manifest after 20 weeks gestation but there is also the existence of post-partum preeclampsia where the symptoms are observed after delivery.

Preeclampsia is defined as a hypertensive disorder in human pregnancy with a blood pressure of $\geq 140/90$ mmHg accompanied with proteinuria exceeding 300mg/24 hours in a previously
normotensive woman after 20 weeks gestation but both symptoms normalize by 12 weeks postpartum (Watanabe et al. 2004; Wagner 2004). It considered a multisystem disorder because of its influence on the following; the central nervous system, the hepato-biliary system, the reproductive system, the renal system, the immune system, the hemodynamic and the vascular system. This condition is peculiar to human pregnancy (Pennington et al. 2012).

2.2 PREVALENCE, MORBITY AND MORTALITY FROM PREECLAMPSIA

The exact epidemiology of preeclampsia is still in doubt because of the differences in definition and diagnostic inaccuracies (Walker 2000). This is further complicated by the different methods used to calculate some of these epidemiological indices (Şahin & Gülmezoglu 2003). It has therefore become very difficult if not impossible to unite these indices to obtain the world accepted epidemiological indices for preeclampsia.

The estimated incidence of preeclampsia is 5%-8% (American College of Obstetricians and Gynecologists 2013). The incidence value differs between the developed and developing worlds (ACOG 2002). The difference in prevalence between the two worlds is due to the following factors; geographic, social, economic and racial differences (Şahin & Gülmezoglu 2003; Lopez-Jaramillo et al. 2001). These factors can result in a 3-fold increase in the incidence in preeclampsia in some populations (Lopez-Jaramillo et al. 2001).

There are a myriad of factors that predisposes someone to preeclampsia. Preeclampsia is usually associated with primigravida (ACOG 2002). Maternal history of preeclampsia increases the risk to between 2 to 5-folds (Uzan et al. 2011). Other risk factors include; chronic hypertension, pregestational diabetes, vascular and connective tissue disease, nephropathy, obesity, African race, age ≥35 years, twin or molar pregnancy and fetal congenital abnormality (Uzan et al. 2011;
ACOG 2002). The incidence of preeclampsia in high altitude populations has been found to be high due to placental hypoxia, smaller uterine artery diameter, and lower uterine artery blood flow (Uzan et al. 2011).

Preeclampsia is not only a disorder of the pregnant state but also causes other chronic and life-threatening conditions in both the mother and the baby. Women with the history of preeclampsia are likely to develop premature cardiovascular disease, such as chronic hypertension, ischemic heart disease, and stroke, later in life (Uzan et al. 2011). Apart from the preterm deliveries with underweight babies associated with preeclampsia, the children of preeclamptic pregnancies may also suffer from stroke, coronary heart disease, and metabolic syndrome in adult life (Uzan et al. 2011).

Preeclampsia is one the most dangerous pregnancy conditions worldwide. It is able to cause an upsurge in the maternal mortality rate by a factor of five and the economic cost to third-world countries is unbearably enormous (Lopez-Jaramillo et al. 2001). About 10% to 15% of the maternal deaths occur as a result of preeclampsia/eclampsia (Uzan et al. 2011).

2.3 CLASSIFICATION OF HYPERTENSIVE DISORDERS OF PREGNANCY

The major cause of maternal mortality worldwide is hemorrhage; however, hypertensive disorders of pregnancy form the number one cause of maternal deaths at the Korle-Bu Teaching Hospital in Ghana (Adu-bonsaffoh et al. 2013). Hypertensive disorders of pregnancy accounts for 31.7% of all maternal mortalities (Adu-Bonsaffoh et al. 2013). Preeclampsia/eclampsia was
found to be the foremost cause of mortality under the hypertensive disorders of pregnancy in the said hospital with 38.1%.

Hypertensive disorders of pregnancy are a spectrum of obstetric conditions characterized by high blood pressure of $\geq 140/90$ mmHg. The hypertensive disorders of pregnancy are classified into:

- Gestational hypertension
- Preeclampsia and eclampsia syndrome
- Preeclampsia syndrome superimposed on chronic hypertension
- Chronic hypertension ((Brown et al. 2009; Tranquilli 2013))

A well-defined classification and stratification according to severity of hypertensive disorders of pregnancy as stated above promote accurate and affective communication among healthcare professionals and also serve as the basis for management (New York State Department of Health 2013).

Gestational hypertension: A new onset of hypertension of $\geq 140/90$ mmHg at $\geq 20$ weeks of gestation in a normotensive subject (Walker 2000). The rise in blood pressure does go without proteinuria and other features of preeclampsia/eclampsia. Pregnancy induced-hypertension is what has been changed to gestational hypertension. Gestational hypertension is a transient disease that usually resolves after 12 weeks post-partum (Mammaro et al. 2009). The diagnosis changes to chronic hypertension when the hypertension does not normalize after 12 weeks post-partum (ACOG, 2013). There is also a high probability of gestational hypertension developing into preeclampsia and can cause perinatal complication and hence requires a strict management module (New York State Department of Health 2013).
Chronic hypertension occurs in a situation where the rise in blood pressure predates conception or occurs before 20 weeks gestation. This condition is usually without proteinuria and other peculiar symptoms of preeclampsia. The hypertension persists beyond 12 weeks post-partum.

Preeclampsia syndrome superimposed on chronic hypertension: this is where preeclampsia occurs in an already hypertensive patient (National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy 2000). In this case the hypertension may occur before conception or early in conception followed by proteinuria after 20 weeks gestation (American College of Obstetricians and Gynecologists 2013). The maternal or fetal complications are usually more severe than either of the two cases (American College of Obstetricians and Gynecologists 2013). The proteinuria usually resolve after 12 weeks post-partum with the hypertension persisting beyond.

2.4 PATHOPHYSIOLOGY OF PREECLAMPSIA

The underlying pathophysiology of preeclampsia is not yet fully clear but is presented in theories that need proving (Mushambi et al. 1996). What is understood about preeclampsia is that the disease stems from an abnormality in the placenta, in that delivery of the placenta resolves the disease condition and molar pregnancy is associated with severe form of the disease (Hladunewich et al. 2007). Also, pathological examination of diseased placentas reveals placental infarct, sclerotic narrowing of arteries and arterioles, diminished cytotrophoblast invasion and abnormal uterine spiral artery remodeling (Hladunewich et al. 2007). The maternal features of preeclampsia include vasospasm, activation of the coagulation system, and perturbations in many humoral and autacoid systems related to volume and blood pressure.
control which promote the overt symptoms of the disease (National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy 2000). The maternal characteristics of preeclampsia are caused by ischemia of the placenta, kidney, liver, and brain (National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy 2000).

One of the major symptoms of preeclampsia is hypertension. Preeclampsia is usually associated with an offset of the vasodilatory effect of normal pregnancy and an increase in vascular resistance (The American College of Obstetricians and Gynecologists 2013). Normal pregnancies are associated with hypervolemia and a decreased response to vasoactive peptides and amines (ACOG 2002) whereas preeclamptics become hyper responsive to these hormones (National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy 2000). The ischemia developed in preeclampsia promotes the formation of ischemic proinflammatory/anti-angiogenic factors (Ahmed & Ramma 2015; Ramma & Ahmed 2011). These ischaemic factors on reaching the maternal system cause vascular endothelial dysfunction resulting in enhanced formation of endothelin-1 and superoxide, increased vascular sensitivity to angiotensin II, and decreased formation of vasodilators such as nitric oxide (Palei et al. 2013; Gilbert et al. 2008). A cumulative effect of all these factors result in the hypertension observed in these patients.

The function of the renal system is normally increased in normal pregnancies (National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy 2000). The converse occurs in preeclampsia, thus an abnormal renal function is observed in these patients as a result a renal lesion that causes hypertrophy of the intracapillary cells. The is a resultant decrease in renal blood flow, decreased glomerular filtration rate and proteinuria (Mirza
The reduced glomerular filtration rate is responsible for the increased blood urea nitrogen (BUN) and creatinine to the level of non-pregnant women (Müller-Deile & Schiffer 2011). Podocyte injury of the glomerulus causes the secretion of specific podocyte protein in the urine leading to proteinuria (kandi et al. 2014). Preeclampsia causes impaired sodium excretion, hypocalciuria, reduced level of 1, 25-dihydroxyvitamin D and increased plasma levels of parathyroid hormone. The cause of the impaired sodium excretion is not clear but is thought to be the reason for the suppression of the diluent-angiotensin system not the other way round.

The hepatic pathological changes during preeclampsia include periportal hemorrhages, ischemic lesions, and fibrin deposition (National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy, 2000). The hepatic damage is usually associated with a development of pain in right upper quadrant or epigastric pain (kandi et al. 2014). The levels of Alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase are raised with no apparent physiological role of the raised alkaline phosphatase (Kandi et al. 2014; ACOG 2002). HELLP (hemolysis, elevated liver enzymes, and low platelet count) syndrome occur in very severe state of the disease (National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy 2000).

The observed neurologic signs of preeclampsia are headache, cortical blindness, scotomata, hyperreflexia and seizure (Cipolla et al. 2011). The seizures differentiate eclampsia from preeclampsia. The cerebral/neurologic manifestations of preeclampsia is because the hypertension cause cerebral autoregulatory dysfunction, blood-brain barrier (BBB) disruption, and passage of damaging protein and serum constituents into the brain (Cipolla et al. 2011).
2.5 THEORIES ON THE ETIOLOGY OF PREECLAMPSIA

Over the centuries of existence of preeclampsia, researchers have promulgated theories in an effort to explain the pathogenesis of the condition. Most of these theories have been disproved with a few left to be disproved or otherwise. Despite the disappointment of the scientific community with the failure of these theories, a huge load of insights have been gleaned to give better understanding of the disease. Endothelial dysfunction has been established as central feature that promote the manifestation of disease symptoms. The theories proposed try to mechanistically explain how a factor/phenomenon is able to cause the development of endothelial dysfunction leading the disease manifestation.

The first theory was based on abnormal spiral artery remodeling as part of the process of placentation. The abnormal process of placental development; result in placental insufficiency and the release of excessive placental materials into the maternal system (Roberts & Hubel 2009). This theory is what has been termed as the two-stage model of preeclampsia. The two-stage model states that, the first stage of preeclampsia development is the development of an asymptomatic under perfused placental that then lead to the second stage clinical manifestation of the disease (Roberts & Hubel 2009). Mechanistically, this theory proposed that the abnormal spiral artery remodeling leads to placental hypoxia, oxidative stress which then trigger an inflammatory response (Ramma & Ahmed 2014). This theory was accepted for years as an explanation of the pathogenic development of preeclampsia until its disproval recently. The theory was abandoned because it was discovered that abnormal spiral artery remodeling is not peculiar to preeclampsia but also observed in conditions such as placental abruption, preterm premature rupture of membrane and intrauterine fetal death (Ramma & Ahmed 2014). Although,
abnormal spiral artery remodeling is observed in each of these conditions, there was the absence of the peculiar of preeclampsia. Additionally, knockout mice models without the spiral artery remodeling process did not exhibit preeclampsia-like symptoms (Burke & Karumanchi 2013). This means the abnormal artery remodeling is not the cause of defective placental growth observed in preeclampsia.

Elevation of the maternal inflammatory response was also proposed as the cause of preeclampsia. This theory was also later disproved because it was not supported by cytokine data and also elevation of the pro-inflammatory factors do not precede preeclampsia onset (Djurovic et al. 2002). Also, the level of inflammatory cytokines produced in preeclampsia does not correlate with disease severity.

The last hypothesis implicates imbalance of anti-angiogenic factors as the cause of preeclampsia. This theory hinges on the fact that a much higher increase in the levels of vascular endothelial growth factor receptor-1 (VEGFR-1) than the vascular endothelial growth factor (VEGF) result in the VEGFR-1 complexes with VEGF denying the vascular system the beneficial angiogenic effect of VEGF (Ahmed et al. 1997). The anti-angiogenic imbalance is strongly associated with preeclampsia onset and disease severity (Ahmed & Ramma 2015). It has also been established that the levels of anti-angiogenic factors increase before clinical manifestation of preeclampsia and also rodent models of preeclampsia can be created by level of VEGF (Levine & Karumanchi 2011; Rana et al. 2011; Ramma & Ahmed 2011).

### 2.6 CLASSIFICATION OF PREECLAMPSIA

Preeclampsia is categorized to mild and severe preeclampsia or early and late on-set preeclampsia based on the severity or gestational age of on-set of the condition. However, the
differences between them are somehow debatable (Steegers et al. 2010). Mild preeclampsia is defined as elevated blood pressure (140/90 mmHg or greater on two measurements that are obtained six or more hours apart, with proteinuria equal +1 on urine dipstick testing while severe preeclampsia is defined as blood pressure ≥160/100 mmHg with greater than +1 urine dipstick testing or ≥140/90 mmHg on two occasions 6 hours apart and anyone of the following: platelets <120,000/ml, aspartate aminotransferase (AST) >45 U/L, alanine aminotransferase (ALT) >60 U/L, and/or creatinine ≥1 mg/dl (Rahman & Ahmed 2015). However, the American College of Obstetricians and Gynecologist advises against this characterization because it is misleading (The American College of Obstetricians and Gynecologists 2013). In the absence the severe features as defined above the mortality and morbidity is significant high hence the recommendation for the use of preeclampsia instead of “severe and mild preeclampsia”. The early on-set preeclampsia develops before gestational week 34, whereas, late on-set preeclampsia develops at or after gestational week 34 (Raymond & Peterson 2011). The presenting features of the two classes are similar; however, they are associated with different maternal and fetal outcomes, biochemical markers, heritability, and clinical features (Raymond and Peterson, 2011).

2.7 DIAGNOSIS OF PREECLAMPSIA

The diagnosis of preeclampsia still remains a challenge even in severe disease condition it can remain asymptomatic and hence the high mortality. The difficulty in diagnosing preeclampsia is because of the absence of a specific marker that can be assayed to give the preeclampsia status of a patient. The symptoms of preeclampsia are used as diagnostic markers which does not give the accurate diagnosis in most situations because of the similarity to other pregnancy related
diseases. The diagnostic criteria are a hypertension of ≥140/90 mmHg and a proteinuria of ≥300mg/24h after gestation week 20 (ACOG 2002).

The ACOG in their 2013 report advised that in situations where the proteinuria is not up to the threshold value, features such as new onset thrombocytopenia, impaired liver function, renal insufficiency, pulmonary edema and visual or cerebral disturbance could be used in addition to hypertension to diagnose preeclampsia (The American College of Obstetricians and Gynecologists 2013). Previous diagnostic methods used edema as a marker of preeclampsia but have now been abandoned because of its presence in other pregnancy conditions (Turner 2010).

2.8 ENDOTHELIAL DYSFUNCTION

The endothelium is the inner thin layer of squamous cells lining the internal surface of organs and cavities of the body, especially the blood vessels, heart, and lymphatic vessels. The functions of the endothelium include fluid filtration, blood vessel tone, homeostasis, neutrophil recruitment, and hormone trafficking. The endothelium lines the entire surface of the circulatory system from the heart to the capillaries.

An endothelial dysfunction is where the endothelium shift from normal function towards reduced vasodilation, a proinflammatory state, and prothrombic properties (Endemann & Schiffrin 2004). A pathologic endothelium or dysfunctional endothelium is the cause of most cardiovascular conditions such as hypertension, coronary artery disease, chronic heart failure, peripheral artery disease, diabetes, and chronic renal failure (Endemann & Schiffrin 2004). Endothelial dysfunction is caused by diabetes or metabolic syndrome, hypertension, smoking, and physical inactivity. Mechanistically, it is believed that oxidative stress cause a reduced NO, increase vasoconstrictors, reduce vasodilators, increased influx of cytokines and adhesion
molecules and increased pro-inflammatory and anti-angiogenic factors (Rajendran et al. 2013). The increase in free radicals or oxidative stress is as a result of the following factors: obesity, smoking, sleep deprivation, acute microbial infections, high glucose intake, and exposure to metals and air pollutants (Rajendran et al. 2013).

2.9 LEPTIN

Leptin is a nonglycosylated peptide hormone encoded by the murine ob gene and the human LEP gene homolog (Lago et al. 2007). It is mainly produced by adipocytes and in small quantities by gastric fundic epithelium, placenta, skeletal muscle, mammary epithelium, intestines and the brain (Yadav et al. 2013). The human leptin is a 16 kDa protein with 167 amino acid and the gene is located on chromosome 7 (Smith et al. 1995; Blüher & Mantzoros 2009; Ahima & Flier 2000). The level of circulating leptin is directly proportional to the white adipose tissue mass (Lago et al. 2007). Leptin belongs to the class 1 cytokine superfamily with closed bundle of four helices in a left-handed twist, with two crossover connections (Lago et al. 2009).

The main function of leptin in the body is to decrease food intake and increase energy expenditure through an induction of anorexigenic reaction and inhibition orexigenic reaction (Lago et al. 2007). Other functions this important factor performs in the body include, regulation of bone mass and secretion of hypothalamo-pituitary-adrenal hormones, increases basal metabolism, influences reproductive function, regulates pancreatic beta-cell function and insulin secretion, is a pro-angiogenic for endothelial cell and affects innate and adaptive immunity (Kielar et al. 1998).
The signaling pathway of leptin uses the leptin receptor with six isoforms produced through alternative splicing. The isoforms of leptin receptor are OB-Ra to OB-Rf classified into soluble form (OB-Re), short form (OB-Ra, OB-Rc, OB-Rd and OB-Rf) and the long form (OB-Rb) (Imagawa et al. 1998; Sweeney 2002). The soluble isoform of leptin receptor serves as binding protein for leptin with long and short forms named because of the differences in length of their transmembrane domains (Sweeney, 2001). The signaling of leptin goes through many pathways including Janus Kinase/signal transducer and activator of transcription (JAK/STAT) pathway, mitogen activated protein kinase (MAPK) pathway, phosphatidylinositol 3-kinase-insulin receptor substrate (PI 3K-IRS) pathway, nitric oxide signaling pathway, phospholipase C (PKC) pathway and cyclic AMP pathway (Sahu 2003). The JAK/STAT and PI 3-Kinase pathways control the food intake regulation action of leptin.

Leptin is released in a pulsatile manner with the peaks and lows following a circadian rhythm (Sinha et al. 1996). The peak is registered at mid-night and early morning with the nadir at mid-day (Sinha et al. 1996). The peak of leptin at night suppress appetite during sleep and also, the night is associated with less physical activity which can result in the accumulation of more calories which leptin will aid in the oxidative process.

Women of comparable weight to their fellow men have higher leptin level than the men. The reason for this variation in leptin level has been thought to be as a result of the rate of leptin production and the white adipose tissue mass (Hellström et al. 2000). Reproductive hormones have also been speculated to play role in the higher leptin level in women than men in that post menopause is associated with a reduced leptin level (Centoa et al. 1999).

Leptin like any other signaling molecule causes disease condition in abnormal levels. The deficiency of this cytokine is associated with obesity, hyperphagia, hyperinsulinemia, and
hyperlipidemia, reproductive and immune dysfunction. The excess of this cytokine is associated with hypertension, obesity, heart disease and stroke, blood sugar imbalance and decreased fertility. The abnormalities in hyperleptinemia are also found in leptin deficiency in that the conditions in excess leptin are as a result of leptin resistance. Hypertension happen in chronically elevated leptin environment through its stimulation of the sympathetic nervous system and deactivation of the vasodilators such a nitric oxide dependent vasodilation and natriuresis (Beltoski et al. 2010).

2.10 LEPTIN IN PREGNANCY

Leptin plays important roles in reproduction. Mechanisms regulating puberty onset in females and fertility in males and females are under the influence of leptin. The regulation of reproduction is through the influence of leptin on gonadotrophin releasing hormone. Pregnancy is a state of change in the hormonal function in an organism that affects other systemic functions such as immune and metabolic functions. Leptin levels increase very early in pregnancy and is seen to facilitate implantation, regulate placental growth, induces the production of human chorionic gonadotrophin from trophoblast cells, promotes mitogenesis and enhances amino acid uptake (Miedle et al. 2012). These functions of leptin in pregnancy are very crucial that pregnancy cannot progress without the effect of leptin.

2.11 LEPTIN IN ANGIOGENESIS

The only theory existing now that tries to explain the pathogenesis of preeclampsia is based the imbalance of angiogenic and antiangiogenic factors. The imbalance inhibits VEGF from
repairing the damaged endothelial tissue resulting in an endothelial dysfunction, the telling pathology in preeclampsia. The imbalance in the angiogenic factors is caused by very high level of VEGF receptor-1 (VEGFR-1) also known as fms-like tyrosine-dependent receptor-1 (Flt-1) that complexes VEGF and hence inhibits angiogenesis (Ahmed & Ramma 2015).

Leptin has been found to be an important regulator of angiogenesis (Islami et al. 2003). Leptin promotes the release of VEGF indirectly through its influence on the human chorionic gonadotropin (hCG) (Islami et al. 2003). It has now been established as an angiogenic factor that regulates vascular fenestration to promote vascular permeability and synergistically induce angiogenesis together with VEGF and fibroblast growth factor (FGF) (Cao et al. 2001; Gonzalez-perez et al. 2010). Cao et al. found out that leptin and VEGF or leptin and FGF combinations resulted in an angiogenic effect much greater than their individual effects or their additive effects.

Park et al. found that leptin stimulation of the release of VEGF and FGF is cell specific (Park et al. 2001). They found that leptin increased the release of VEGF in the human umbilical vein endothelial cells in a dose-dependent manner, but had little effect on FGF in the same cell type. A further analysis of the effect of leptin on the release of VEGF and FGF in human coronary artery smooth muscle cells by the same group showed little influence.

### 2.12 LEPTIN IN PREECLAMPSIA

Preeclampsia is a multifactorial disease with heightened hypoxia, exaggerated oxidative stress and abnormal inflammatory state (Ahmed & Ramma 2015). Hypoxia, oxidative stress and the abnormal inflammatory state are normally associated with increased leptin level (Lago et al. 2009; Netzer et al. 2015). Leptin through its angiogenic function facilitate capillary fenestration to improve circulation and resolve the hypoxic state. Oxidative stress in preeclampsia contribute
to endothelial dysfunction (Ahmed & Ramma 2015). Leptin, together with other angiogenic factors, repair the injured endothelium.

Leptin has been reported to cause hypertension (Taylor et al. 2015; Molvarec et al. 2011). Leptin causes an increase in blood pressure by activating the sympathetic nervous system and nitric oxide synthesis (Molvarec et al. 2011). The hypertension in preeclampsia is precipitated by the same sympathetic activation and a complex interplay of other factors (Molvarec et al. 2011). It is therefore possible; leptin plays a part in the development of preeclampsia symptoms.

Other research works have reported an increase in leptin level in preeclampsia (Anim-Nyame et al. 2000). The elevated leptin in preeclampsia is believed to facilitate the availability and delivery of nutrients to the fetus. There are other works that have reported no change in leptin level between preeclamptic subjects and pregnant controls (Kafulafula et al. 2002). These results are possibly due to the type of leptin assayed in the study; whether free leptin or total leptin. Leptin, like any other endocrine/paracrine molecule, has binding proteins in the blood that influence the level of free leptin in the blood. Higher levels of these binding proteins result in a reduced leptin level. Another possible explanation for the difference in leptin levels in preeclampsia is race, because most the studies done so far used Caucasians or Asians except the work by Kafulafula and colleagues that was done in Africa. Some works have implicated gestational age at recruitment as a cause of the different leptin levels (Salomon et al. 2003). Leptin exhibits a circadian cycle of lows and peaks and therefore influenced by the time of the day the sample is taken. The conflicting results presented on the levels of leptin in preeclamptics and normotensive subjects could be due to the difference in the time of day the samples were collected.
CHAPTER THREE

METHODOLOGY

3.1 STUDY SITE

The Obstetrics and Gynecology Department of the Korle Bu Teaching Hospital served as the study site. The study took place from April to June of 2016. The department performs about 12,000 deliveries a year with most of the patients being referral cases. The department can boast of the finest obstetricians, gynecologist and mid-wives that provide the necessary care for patients from all over Ghana and even from the West African sub-region. Antenatal and all other obstetric treatment form the care package the department provides.

The antenatal clinic operates from Monday to Friday 8:30 am to 5 pm with the maternity and labor wards opened 24 hours each day. Pregnant women visiting the antenatal clinic go through routines such as weight measure, blood pressure measurement, urinalysis for protein and glucose, HIV and hepatitis C test and counseling and the tetanus vaccination and malaria treatment. The pregnant women are also educated on how to take good care of themselves and their babies during pregnancy and after delivery.

3.2 STUDY DESIGN

A case control study design was used. The study subjects were pregnant women with preeclampsia and age and gestational age matched normotensive pregnant women.

3.3 CASE DEFINITION

The American College of Obstetricians and Gynecologists’ (ACOG) criteria was used for preeclampsia diagnosis. Accordingly, diastolic blood pressure above 90 mmHg and systolic blood pressure above 140 mmHg measured in sitting position at least for two times after a 15 minutes rest and in at least two consecutive measurements, six hours apart, and proteinuria of
300 mg or more in a 24 hour urine collection or ≥ 1+ dipstick on a random urine sample was considered as preeclampsia. Preeclampsia is sub-classified based on its severity or time of on-set. The early on-set preeclampsia occur prior to 34 weeks of gestation while late on-set preeclampsia from 34 weeks onwards. Severe preeclampsia is defined by a blood pressure of ≥160/100 mmHg and a > +1 proteinuria on a urine dipstick whereas mild preeclampsia is defined as a blood pressure of ≥140/90 or <160/100 mmHg with a +1 proteinuria.

3.4 ELIGIBILITY CRITERIA

3.4.1 INCLUSION CRITERIA
The study recruited pregnant women with or without preeclampsia within the age groups of 16 to 45 years. The recruited subjects had gestational ages 16 weeks and above. Consenting to take part in the study formed part of the inclusion criteria.

3.4.2 EXCLUSION CRITERIA
Refusal to participate in the study was the number one exclusion criteria. Pregnant women with conditions such as chronic hypertension, renal disease, diabetes mellitus, urinary infection, cardiovascular diseases, thyroid dysfunction, obesity and infectious diseases were excluded from the study.

3.5 SAMPLE SIZE DETERMINATION

The sample size for this study was computed from the formula:

\[ n = \left( \frac{r+1}{r} \right) \frac{\sigma^2 (Z_\beta + Z_{\alpha/2})^2}{(difference)^2} \]
Where \( Z_{\alpha}, 1.96 \) is the standard score for the confidence interval of 95%, \( Z_{\beta}=0.84 \) for 80% power, \( r=1 \) for control/case ratio of 1:1, \( \sigma=16.13 \) and the “deference”=8.065 is half the standard deviation (Rahman et al., 2015).

\[
n = \left( \frac{1 + 1}{1} \right) \frac{(16.13)^2(0.84 + 1.96)^2}{(8.065)^2}
\]

\[n = 62.72\]

This gave a minimum sample size of 63. Therefore, a sample size of 130 was used in the study comprising 65 normotensive pregnant and 65 women with preeclampsia.

### 3.6 BLOOD SAMPLE COLLECTION AND PROCESSING

A vacutainer needle was used to take the blood samples under aseptic conditions. The needles were used once and disposed of. About 8 ml of blood was taken from the antecubital area. The sample was divided into two, 4 ml into an EDTA tube and 4 ml into a gel tube. The sample in the EDTA tube was used for hematological analysis. The gel tube sample was centrifuged at 5000g for 5 min and separated. Part of the serum from the gel tube was used for analysis of the biochemical parameters. The remaining part was stored at -81 °C and later used for leptin ELISA analysis.
3.7 LEPTIN ENZYME-LINKED IMMUNOSORBENT ASSAY

The serum levels of leptin were assayed using the R&D Duo set enzyme linked immunoassay (ELISA) kit. The kit uses the sandwich ELISA method.

A volume of 1 μL of the serum samples was added to 199 μL of regent diluents to achieve 1:200 dilutions. The samples were then stored in a refrigerator at a temperature of 2ºC and used for the assay within one week.

   a. Plate Coating with Capture Antibody: Anti-human leptin capture antibody standard was reconstituted with 1.0 mL of PBS to achieve a concentration of 180 μg/mL. It was further diluted down in PBS to a working concentration of 1.0 μg/mL. Aliquots of 100 μL of the diluted capture antibody solution were used to coat a 96-well microplate and incubated overnight at room temperature.

   b. After the overnight incubation, the plates were washed three times with wash buffer and blotted dry.

   c. Aliquots of 300 μL of reagent diluents was added to each well and incubated for 1 hour for blocking.

   d. The microplates were washed three times with wash buffer after blocking and blotting done.

   e. Aliquots of 100 μL of the diluted samples and the reconstituted leptin standards were added to wells in the microplates. The sample or specific standard concentration added to a well was noted. The plates were sealed with plate sealers and incubated for 2 hours at room temperature.

   f. After the 2-hour incubation period, the plates were washed three times and blotted.
g. A volume of 100 μL of detection antibody, diluted in reagent diluents with normal goat serum was added to each well and incubated for 2 hours at room temperature.

h. The plate were washed three times and blotted afterwards.

i. A volume of 100 μL of diluted Streptavidin-HRP was added to each well, covered and incubated for, at room temperature, for 20 minutes.

j. The microplates were washed three times and blotted.

k. Aliquots of 100 μL of substrate solution were added to each well and incubated for 20 minutes at room temperature in darkness.

l. The reaction was stopped by the addition of a volume of 50 μL of stop solution to each well.

m. A microplate reader was used to obtain the optical densities at 450 nm.
CHAPTER FOUR

RESULTS

4.1 SUBJECTS’ GENERAL CHARACTERISTICS
A total of 132 consented pregnant women with an average age of (29.20 ± 6.19) years were enrolled into the study with 68 of them being preeclamptics and 64 normotensive pregnant women. The mean age of the normotensive pregnant was found to be (28.51 ± 5.75) years and that for the preeclamptics was determined to be (29.86 ± 6.56) years with an insignificant p-value of 0.2079. Ten participants had no formal education at all representing 6.9% with 77.6% with educational level ranging from basic to tertiary institution comprising of Primary 9%, Junior High School 37.5%, Senior High School 15.3% and 16% with tertiary education while 15.3% decline to answer that question. For those with preeclampsia, 78% had education to at least the basic level. The participants were made up of 75.7% Christians and 9.7% Muslims with the remaining percentage for other sects. As much 78.8% of the preeclamptics were Christians with 12.1% Muslims. Fifty-two point eight percent of the participants were married with 18.8% single. Out the total of 33 preeclamptics 66.7% were married with 12.1% being single.

4.2 ANTHROPOMETRIC CHARACTERISTICS OF STUDY SUBJECTS
Measurements of weight in kilograms, height in metres and body mass index (BMI) (kg/m²) of participants obtained during the study is presented below. Table 4.1 shows the comparison of weight, height and BMI between the preeclamptics and the controls using student t-test. Significant difference between the cases and controls was observed in their weight with no significant difference in their height and BMI. Table 4.2 compares the weight, height and BMI between severe and mild preeclamptics. No significant difference was observed in the weight, height and BMI between the cases and controls. The recruitment systolic and diastolic blood
pressure for the cases were significantly greater than that of the controls with p<0.0001. The severe preeclampsics had greater systolic and diastolic blood pressure at recruitment than the controls.

Table 4.1: Comparison of weight, height and BMI of preeclamptics and pregnant controls

<table>
<thead>
<tr>
<th>parameter</th>
<th>Subject category (mean ±SD)</th>
<th>t-statistic</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>preeclampsia 71.57±15.18(60)</td>
<td>2.9010</td>
<td>0.0044*</td>
</tr>
<tr>
<td></td>
<td>Normotensive pregnant 64.13±12.50(58)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.57±0.08(45)</td>
<td>0.6024</td>
<td>0.5484</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.41±6.46(45)</td>
<td>2.0622</td>
<td>0.0421*</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>163.10±21.29(59)</td>
<td>6.1245</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>104.6±17.83(59)</td>
<td>13.0121</td>
<td>&lt;0.0001*</td>
</tr>
</tbody>
</table>

*p-value<0.05

Table 4.2: Comparison of weight, height and BMI between mild and severe preeclamptics

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>Subject category (mean ±SD)</th>
<th>t-statistic</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>MILD preeclampsia 72.60±14.57 (28)</td>
<td>0.1883</td>
<td>0.8513</td>
</tr>
<tr>
<td></td>
<td>SEVERE PREECLAMPSIA 71.85±15.69 (30)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.56±0.08 (21)</td>
<td>0.0000</td>
<td>1.0000</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.97 ± 1.45 (21)</td>
<td>1.2790</td>
<td>0.2081</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>177.80±15.56(33)</td>
<td>10.1733</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>113.80±17.56(33)</td>
<td>5.2339</td>
<td>&lt;0.0001*</td>
</tr>
</tbody>
</table>

*p-value<0.05
4.4 HAEMATOLOGICAL PARAMETERS FOR PREECLAMPTICS AND NORMOTENSIVE PREGNANT WOMEN

A comparison of the hematological parameters between the preeclamptics and controls showed significant difference in white blood cell count (WBC) ($p=0.0014$), lymphocytes (Lym%) ($p<0.0001$), monocytes (Mon %) ($p=0.0017$), granulocytes (Gra %) ($p=0.0004$), granulocytes count (Gra#) ($p=0.0006$), red blood cell count (RBC) ($P<0.0001$), hemoglobin (HB) ($P=0.0006$), hematocrit (HCT) ($P=0.0063$), mean cell hemoglobin concentration (MCHC) ($P=0.0020$) and mean platelet volume (MPV) ($p=0.0020$) between them with no significant difference in the other hematological parameters as in Table 4.3.
Table 4.3: Comparison of Hematological parameters between preeclamptics and normotensive pregnant women

<table>
<thead>
<tr>
<th>Measured parameter</th>
<th>Subject category (mean ± SD)</th>
<th>PREECLAMPSIA</th>
<th>NORMOTENSIVE PREGNANT</th>
<th>t-statistic</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC(*10^9)/L</td>
<td></td>
<td>9.84±3.93(72)</td>
<td>7.69±3.64(62)</td>
<td>3.2667</td>
<td>0.0014*</td>
</tr>
<tr>
<td>Lym%</td>
<td></td>
<td>19.30±8.83(72)</td>
<td>26.69±7.59(62)</td>
<td>5.1513</td>
<td>&lt; 0.0001*</td>
</tr>
<tr>
<td>Mon%</td>
<td></td>
<td>4.43±2.54(72)</td>
<td>5.97±3.03(62)</td>
<td>3.2005</td>
<td>0.0017*</td>
</tr>
<tr>
<td>gra%</td>
<td></td>
<td>74.31±10.79(47)</td>
<td>67.34±9.15(62)</td>
<td>3.6445</td>
<td>0.0004*</td>
</tr>
<tr>
<td>Lym#(*10^3)/mm^3</td>
<td></td>
<td>2.19±4.82(72)</td>
<td>1.83±0.53(62)</td>
<td>0.5848</td>
<td>0.5600</td>
</tr>
<tr>
<td>Mon#(*10^3)/mm^3</td>
<td></td>
<td>0.38±0.23(72)</td>
<td>0.38±0.20(62)</td>
<td>0.0000</td>
<td>1.0000</td>
</tr>
<tr>
<td>gra#(*10^3)/mm^3</td>
<td></td>
<td>7.85±3.61(47)</td>
<td>5.48±3.40(62)</td>
<td>3.5094</td>
<td>0.0006*</td>
</tr>
<tr>
<td>RBC(10^6)/mm^3</td>
<td></td>
<td>4.56±1.24(72)</td>
<td>3.80±0.56(62)</td>
<td>4.4494</td>
<td>&lt; 0.0001*</td>
</tr>
<tr>
<td>HGB(g/DL)</td>
<td></td>
<td>12.09±2.84(72)</td>
<td>10.46±1.81(62)</td>
<td>3.8890</td>
<td>0.0002*</td>
</tr>
<tr>
<td>HCT %</td>
<td></td>
<td>24.49±18.39(72)</td>
<td>31.16±4.81(62)</td>
<td>2.7740</td>
<td>0.0063*</td>
</tr>
<tr>
<td>MCV(*10^-6)m^3</td>
<td></td>
<td>82.14±14.63(72)</td>
<td>82.60±10.15(62)</td>
<td>0.2081</td>
<td>0.8355</td>
</tr>
<tr>
<td>MCH(pg)</td>
<td></td>
<td>27.48±3.47(72)</td>
<td>27.79±4.36(62)</td>
<td>0.4580</td>
<td>0.6477</td>
</tr>
<tr>
<td>MCHC(g/DL)</td>
<td></td>
<td>32.22±2.86(72)</td>
<td>33.50±1.52(62)</td>
<td>3.1596</td>
<td>0.0020*</td>
</tr>
<tr>
<td>RDW (%)</td>
<td></td>
<td>14.69±3.48(72)</td>
<td>15.04±2.44(62)</td>
<td>0.6637</td>
<td>0.5082</td>
</tr>
<tr>
<td>PLT(*10^3)/mm^3</td>
<td></td>
<td>227.71±100.33(72)</td>
<td>216.79±67.41(62)</td>
<td>0.7271</td>
<td>0.4684</td>
</tr>
<tr>
<td>MPV(*10^-6)m^3</td>
<td></td>
<td>9.30±1.28(72)</td>
<td>8.69±0.82(62)</td>
<td>3.2248</td>
<td>0.0016*</td>
</tr>
<tr>
<td>PCT (%)</td>
<td></td>
<td>0.21±0.08(47)</td>
<td>0.19±0.05(62)</td>
<td>1.6001</td>
<td>0.1125</td>
</tr>
<tr>
<td>PDW (%)</td>
<td></td>
<td>15.06±2.85(72)</td>
<td>14.12±2.99(62)</td>
<td>1.8609</td>
<td>0.0650</td>
</tr>
</tbody>
</table>

WBC, white blood cells; Lym, lymphocytes; Mon, monocytes; Gra, granulocytes; Hb, hemoglobin; HCT, Hematocrit; RBC, red cell count; MCV, mean cell volume; MCH, mean cell hemoglobin; MCHC, mean cell hemoglobin concentration; RDW Red cell distribution width, MPV mean platelet volume, PLT platelets count, PCT platelet crit. PDW platelets distribution width of; WBC white blood cells.*significant at p≤ 0.05
### 4.4 CLINICAL CHEMICAL PARAMETERS AND LEPTIN FOR PREECLAMPTICS AND NORMOTENSIVE PREGNANT WOMEN

From table 4.4 below, a significant difference was observed between preeclamptics and pregnant controls in urea, AST and ALT with p<0.05. The differences in the other parameters were not significant.

**Table 4.4: Comparison of chemistries and leptin level between preeclamptics and controls**

<table>
<thead>
<tr>
<th>parameter</th>
<th>Subject category (mean ± SD)</th>
<th>t-statistic</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PREECLAMPSIA</td>
<td>NORMOTENSIVE PREGNANT</td>
<td></td>
</tr>
<tr>
<td>Na+ (mmol/L)</td>
<td>139.12±3.31(65)</td>
<td>139.66±3.40(65)</td>
<td>0.9175</td>
</tr>
<tr>
<td>K+ (mmol/L)</td>
<td>4.83±6.01(64)</td>
<td>4.04±0.44(65)</td>
<td>1.0570</td>
</tr>
<tr>
<td>UREA (mmol/L)</td>
<td>1.71±0.25(65)</td>
<td>1.84±0.23(65)</td>
<td>3.0853</td>
</tr>
<tr>
<td>CREATININE(µmol/L)</td>
<td>58.86±7.82(65)</td>
<td>60.63±6.79(65)</td>
<td>1.3779</td>
</tr>
<tr>
<td>AST(U/L)</td>
<td>38.14±11.64(64)</td>
<td>29.94±13.63(65)</td>
<td>3.6718</td>
</tr>
<tr>
<td>ALT(U/L)</td>
<td>30.58±12.22(64)</td>
<td>22.37±10.72(65)</td>
<td>4.0581</td>
</tr>
<tr>
<td>LEPTIN(ng/ml)</td>
<td>34.54±40.88(64)</td>
<td>29.22±32.14(63)</td>
<td>-0.8348</td>
</tr>
</tbody>
</table>

AST, aspartate aminotransferase; ALT, alanine aminotransferase; *p*-value <0.05
4.5 HEMATOLOGICAL PARAMETERS FOR MILD AND SEVERE PREECLAMPTICS

The hematological parameters of the severe and mild preeclamptics showed no significant difference between the two categories as in table 4.5 below.
Table 4.5: Comparison of hematological parameters between severe and mild preeclampsia

<table>
<thead>
<tr>
<th>Measured parameter</th>
<th>Subject category (mean ± SD)</th>
<th>t-statistic</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Severe preeclampsia</td>
<td>Mild preeclampsia</td>
<td></td>
</tr>
<tr>
<td>*<em>WBC(<em>10^9)/L</em></em></td>
<td>10.32± 3.85 (33)</td>
<td>9.16± 3.59 (35)</td>
<td>1.2857</td>
</tr>
<tr>
<td><strong>Lym%</strong></td>
<td>19.32± 9.07 (33)</td>
<td>19.55± 7.40 (35)</td>
<td>0.1149</td>
</tr>
<tr>
<td><strong>Mon%</strong></td>
<td>4± 1.93 (33)</td>
<td>4.61± 2.69 (35)</td>
<td>1.0687</td>
</tr>
<tr>
<td><strong>gra%</strong></td>
<td>74.60± 11.11 (21)</td>
<td>74.90± 8.74 (24)</td>
<td>0.1013</td>
</tr>
<tr>
<td>*<em>Lym#(<em>10^3)/mm^3</em></em></td>
<td>2.95± 7.06 (33)</td>
<td>1.59± 0.68 (35)</td>
<td>1.1346</td>
</tr>
<tr>
<td>*<em>Mon#(<em>10^3)/mm^3</em></em></td>
<td>0.35± 0.20 (33)</td>
<td>0.39± 0.25 (35)</td>
<td>0.7258</td>
</tr>
<tr>
<td>*<em>gra#(<em>10^3)/mm^3</em></em></td>
<td>8.29± 3.47 (21)</td>
<td>7.64± 3.67 (24)</td>
<td>0.6079</td>
</tr>
<tr>
<td><strong>RBC(10^6)/mm^3</strong></td>
<td>4.44± 0.89 (33)</td>
<td>4.68± 1.56 (35)</td>
<td>0.7729</td>
</tr>
<tr>
<td><strong>HGB(g/DL)</strong></td>
<td>12.35± 2.36 (33)</td>
<td>11.82± 3.33 (35)</td>
<td>0.7531</td>
</tr>
<tr>
<td><strong>HCT %</strong></td>
<td>22.93± 17.80 (33)</td>
<td>26.72± 18.94 (35)</td>
<td>0.8491</td>
</tr>
<tr>
<td>*<em>MCV(<em>10^-6)m^3</em></em></td>
<td>84.37± 9.10 (33)</td>
<td>82.03± 12.78 (35)</td>
<td>0.8613</td>
</tr>
<tr>
<td><strong>MCH(pg)</strong></td>
<td>27.42± 3.58 (33)</td>
<td>27.50± 3.47 (35)</td>
<td>0.0936</td>
</tr>
<tr>
<td><strong>MCHC(g/DL)</strong></td>
<td>32.45± 1.38 (33)</td>
<td>32.02± 3.89 (35)</td>
<td>0.6002</td>
</tr>
<tr>
<td><strong>RDW (%)</strong></td>
<td>14.45± 2.49 (33)</td>
<td>15.00± 4.36 (35)</td>
<td>0.6336</td>
</tr>
<tr>
<td>*<em>PLT(<em>10^3)/mm^3</em></em></td>
<td>233.61± 99.12 (33)</td>
<td>224.15± 106.88 (35)</td>
<td>0.3778</td>
</tr>
<tr>
<td>*<em>MPV(<em>10^-6)m^3</em></em></td>
<td>9.02± 1.07 (33)</td>
<td>9.51± 1.48 (35)</td>
<td>1.5247</td>
</tr>
<tr>
<td><strong>PCT (%)</strong></td>
<td>0.21± 0.08 (21)</td>
<td>0.21± 0.09 (24)</td>
<td>0.0000</td>
</tr>
<tr>
<td><strong>PDW (%)</strong></td>
<td>14.79± 2.17 (33)</td>
<td>15.19± 3.50 (35)</td>
<td>0.5623</td>
</tr>
</tbody>
</table>

WBC, white blood cells; Lym, lymphocytes; Mon, monocytes; Gra, granulocytes; Hb, hemoglobin; HCT, Hematocrit; RBC, red cell count; MCV, mean cell volume; MCH, mean cell hemoglobin; MCHC, mean cell hemoglobin concentration; RDW Red cell distribution width, MPV mean platelet volume, PLT platelets count, PCT platelet crit. PDW platelets distribution width of; WBC white blood cells. *significant at p≤ 0.05
4.5 CLINICAL CHEMICAL PARAMETERS AND LEPTIN IN MILD AND SEVERE PREECLAMPTICS

There was no significant difference in the clinical chemical parameters observed between the mild and severe preeclamptic subjects with p>0.05 as in Table 4.6.

Table 4.6: Comparison of chemistries and leptin level between mild and severe preeclampsia

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Subject category (mean ± SD)</th>
<th>t-statistic</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mild preeclampsia</td>
<td>Severe preeclampsia</td>
<td></td>
</tr>
<tr>
<td>Na+ (mmol/L)</td>
<td>139± 2.84 (29)</td>
<td>139.34± 3.94 (29)</td>
<td>0.3770</td>
</tr>
<tr>
<td>K+ (mmol/L)</td>
<td>4.05± 0.50 (29)</td>
<td>4.07± 0.52 (28)</td>
<td>0.1480</td>
</tr>
<tr>
<td>UREA (mmol/L)</td>
<td>1.70± 0.26 (29)</td>
<td>1.72± 0.26 (29)</td>
<td>0.2929</td>
</tr>
<tr>
<td>CREATININE(µmol/L)</td>
<td>58.10± 4.66 (29)</td>
<td>60.72± 9.20 (29)</td>
<td>1.3681</td>
</tr>
<tr>
<td>AST(U/L)</td>
<td>37.48± 9.96 (29)</td>
<td>37.86± 11.22 (28)</td>
<td>0.1353</td>
</tr>
<tr>
<td>ALT(U/L)</td>
<td>29.93± 11.19 (29)</td>
<td>30.29± 10.53 (28)</td>
<td>0.1250</td>
</tr>
<tr>
<td>LEPTIN(ng/ml)</td>
<td>31.55±23.79 (32)</td>
<td>27.66±21.50 (30)</td>
<td>-0.6767</td>
</tr>
</tbody>
</table>

AST, aspartate aminotransferase; ALT, alanine aminotransferase
4.6 CORRELATION OF SYSTOLIC BLOOD PRESSURE AT RECRUITMENT AND LEPTIN LEVEL IN PREECLAMPTICS

In trying to establish any relationship between leptin and systolic blood pressure, a graph of correlation was constructed as in Fig. 4.1 below. The figure showed a weak negative correlation but was found to be insignificant relation with $p=0.2453$ and $R^2=0.02491$.

**Fig. 4.1: Correlation of systolic blood pressure at recruitment and leptin level in preeclamptics.**
4.7 THE RELATIONSHIP BETWEEN DIASTOLIC BLOOD PRESSURE AT DIAGNOSIS AND LEPTIN LEVEL IN PREECLAMPTICS

With no relation between leptin and systolic blood pressure in the preeclamptics, a similar test of relatedness was conducted for leptin and diastolic blood pressure in the same preeclamptics. This also showed an absence of a significant relationship as fig. 4.2 with \( p = 0.3083 \) and \( R^2 = 0.0191 \).

Fig. 4.2: Correlation of diastolic blood pressure at diagnosis and leptin level in preeclamptics
4.8 CORRELATION OF SYSTOLIC BLOOD PRESSURE AT DIAGNOSIS AND LEPTIN LEVEL IN SEVERE PREECLAMPSIA SUBJECTS

A further test of relatedness was conducted between leptin and systolic blood pressure at diagnosis in severe preeclamptics. This also showed the absence of significant relation between the two parameters in severe preeclamptics with $p=0.2737$ and $R^2=0.04263$.

Fig. 4.3: Correlation of systolic blood pressure at diagnosis and leptin level in severe preeclampsia subjects
4.9 CORRELATION OF DIASTOLIC BLOOD PRESSURE AT DIAGNOSIS AND LEPTIN LEVEL IN SEVERE PREECLAMPTICS

There was no significant association between the diastolic blood pressure and leptin level in severe preeclamptics with $p=0.1332$ and $R^2=0.07869$.

Fig. 4.4: Correlation of diastolic blood pressure at diagnosis and leptin level in severe preeclampsia

![Graph showing correlation between diastolic blood pressure and leptin level]
4.10 ASSOCIATION OF SYSTOLIC BLOOD PRESSURE AT DIAGNOSIS AND LEPTIN LEVEL IN MILD PREECLAMPTICS

In relating systolic blood pressure at diagnosis to leptin level in mild preeclamptics found no significant association between the two parameters with \( p = 0.0891 \) and \( R^2 = 0.1258 \).

Fig. 4.5: Correlation of systolic blood pressure at diagnosis and leptin level in mild preeclampsia subjects

\[
P = 0.0891 \\
R^2 = 0.1258
\]
4.11 ASSOCIATION OF DIASTOLIC BLOOD PRESSURE AT DIAGNOSIS AND LEPTIN LEVEL IN PREECLAMPTICS

A side by side analysis of diastolic blood pressure at diagnosis and leptin level in mild preeclamptics showed the absence of a significant association with $p=0.2342$ and $R^2=0.06369$.

Fig. 4.6: Correlation of diastolic blood pressure at diagnosis and leptin level in mild preeclampsia
4.12 ASSOCIATION OF LEPTIN LEVEL TO BOOKING SYSTOLIC BLOOD PRESSURE INPREGNANT CONTROLS

There was also no significant association between booking systolic blood pressure and leptin level with $p=0.1917$ and $R^2=0.03080$.

*Fig. 4.7: Correlation of systolic blood pressure at booking and leptin level in normotensive pregnant subjects*
4.13 THE RELATIONSHIP BETWEEN DIASTOLIC BLOOD PRESSURE AT BOOKING AND LEPTIN LEVEL IN PREGNANT CONTROLS

The graph of regression between diastolic blood pressure at booking and leptin level in normotensive pregnant subjects showed no significant relationship between the two parameters as in Fig 4.7.

Fig. 4.8: Correlation of diastolic blood pressure at booking and leptin level in normotensive pregnant subjects
4.14 COMPARISON OF GESTATIONAL AGE OF PREECLAMPTICS AND PREGNANT CONTROLS

The booking gestational age for preeclamptics (17.20 ± 5.94) weeks and pregnant controls (15.53 ± 5.71) weeks as in the Fig. 4.17 was found not to be significantly different with p=0.1642.

Fig. 4.9: Booking gestational ages (GA) of preeclamptics (PE) and pregnant controls (PC)
4.15 BOOKING GESTATIONAL AGE OF MILD AND SEVERE PREECLAMPSIA

A comparison of the booking gestational ages between the mild and severe preeclamptics showed no significant difference with \( p = 0.9081 \) as in fig. 4.9 below. The mean booking gestational ages for mild preeclamptics and severe preeclamptics were found to be \( (17.29 \pm 5.60) \) weeks and \( (17.06 \pm 6.77) \) weeks respectively.

Fig. 4.10: Booking gestational age (GA) of mild and severe preeclamptics
4.16 RECRUITMENT GESTATIONAL AGE OF CASES AND CONTROLS

There was a significant difference between the recruitment gestational ages in weeks for mild (36.00 ± 3.20) and severe preeclamptics (33.34 ± 5.02) with p=0.032 as in fig. 4.10 below.

Fig. 4.11: Diagnostic gestational age (GA) of mild and severe preeclamptics
4.17 DELIVERY GESTATIONAL AGE OF CASES AND CONTROLS

The delivery gestational age for pregnant controls (37.37 ± 2.09) weeks was found to be significantly higher than that of the preeclamptics (35.14 ± 3.67) weeks with p=0.0015 as in fig. 4.11.

Fig. 4.12: Delivery gestational age for preeclamptics and pregnant controls
4.18 DELIVERY GESTATIONAL AGE OF MILD AND SEVERE PREECLAMPSIA

The delivery gestational age of the mild preeclamptics (37.34 ± 2.14) weeks was also found to be significantly higher than that of the severe preeclamptics (35.57 ± 3.78) weeks with p=0.0096 as in fig. 4.13 below.

Fig. 4.13: Delivery gestational age of mild and severe preeclampsia
4.19 BIRTH WEIGHT OF CASES AND CONTROLS

A comparison of the birth weight of babies delivered by preeclamptics (2.12 ± 0.92) kg and pregnant controls (3.01 ± 0.78) kg showed a significant difference between the two with \( p=0.0003 \)

Fig. 4.14: Birth weight of preeclamptics and pregnant controls

![Birth weight of preeclamptics and pregnant controls](image-url)
4.20 BIRTH WEIGHT OF MILD AND SEVERE PREECLAMPSIA

A comparison of birth weight of delivered babies between mild and severe preeclampsia showed that babies delivered by mild preeclamptics (2.54 ± 0.67)kg were found to have significantly higher weights than the babies delivered by the severe preeclamptics (1.86 ± 0.97)kg with p=0.0285.

Fig. 4.15: Birth weight of mild and severe preeclampsia

![Birth weight of mild and severe preeclampsia](image-url)
CHAPTER FIVE
DISCUSSION AND CONCLUSION

5.1 DISCUSSION

The study age-matched the preeclamptics with the controls. The booking weight of the preeclamptics was found to be significantly greater than the booking weight of the pregnant controls. This confirms the evidence that a sudden weight gain is a predisposing factor to getting preeclampsia (Rahman & Ahmed 2015; The American College of Obstetricians and Gynecologists 2013). The higher weight in preeclamptics than the normotensive pregnant is thought to be due the edema which is symptom of preeclampsia (Asnafi et al. 2011). Obesity is associated with an increased production of reactive oxygen species that cause an endothelial dysfunction the pathophysiological feature in preeclampsia (Adya et al. 2015). There was no significant difference in the height between the preeclamptics and the controls. With no significant difference in height, the BMI of the cases and controls were not significant either. This makes weight the major risk factor of preeclampsia irrespective of the height of the person. Despite weight being a predisposing factor of preeclampsia, it had no influence on the severity of preeclampsia.

5.2 HEMATOLOGICAL AND BIOCHEMICAL INDICES

The white cell indices showed a significantly higher WBC, granulocyte percentage and granulocyte number in preeclampsia than the controls. The lymphocytes and monocytes percentages of the control were greater than the cases. The higher granulocytes in the preeclamptic are as a result of the maladaptation of the immune system in preeclampsia. The higher WBC in the preeclamptic might also be due to the heightened immune action explanation
but other works found no significant difference in WBC between preeclampsia and the controls (Abdullahi et al. 2014; Avcıoğlu et al. 2016). Another reason for difference in WBC levels between the cases and controls is probably due to the different stress levels the two groups are exposed to (Purohit et al. 2015).

The preeclamptic subjects had significantly higher RBC, HGB and MPV than the controls. The controls on the other hand had greater MCHC and HCT than the cases. There was no significant difference in the other red cell parameters. The greater RBC and HGB in the preeclamptic subjects is possibly a physiological mechanism to ensure that oxygen reaches the fetus in the under perfused condition. The studies by Abdullahi et al and Avcıoğlu et al found a significantly higher RDW in preeclamptic subjects than the controls. The RDW has been found to cause hypertension probably through its action on the inflammatory response (Abdullahi et al. 2014; Avcıoğlu et al. 2016).

A further analysis to find out the contribution of the hematological indices on the severity of preeclampsia showed no significant difference in the hematological parameters between the two categories of preeclampsia.

Analysis of the biochemical test parameters showed a significant elevation in AST and ALT in the preeclamptic subjects than the controls. Urea was higher in the controls than the cases with no difference in creatinine, Na⁺ and K⁺ between the two groups. The elevated AST and ALT point to a liver injury with ALT being a more specific indicator than AST (Al-Jameil Noura 2015). The creatinine and urea test the function of the kidneys. Conflicting results have been reported on creatinine and urea in the literature (Manjareeka & Nanda 2013). It has been found that Na⁺ and K⁺ correlates well with blood pressure (Adewolu 2013). No significant difference
was observed in the biochemical test parameters between the mild and severe forms of preeclampsia. The biochemical markers are not indicative of preeclampsia severity.

5.3 LEPTIN LEVELS

This study found no significant difference in serum leptin levels between preecamptics and the controls. A further analysis to assert the role of leptin in preeclampsia severity proved that there was no significant difference in leptin between the mild and severe forms of preeclampsia. The outcome of this study agrees with two other works by Asnafi et al and Salomon et al in which leptin concentration between preeclamptic cases and controls was not significantly different (Salomon et al. 2003; Asnafi et al. 2011). However, this runs counter to other findings in which leptin concentration in preeclamptics was found to be significantly greater than leptin concentration in the pregnant controls (Anim-Nyame et al. 2000; Rahman & Ahmed 2015). The study by Rahman and Ahmed went further to compare the leptin concentration between the mild cases and the severe form of preeclampsia and found a significantly greater concentration of leptin in the severe preeclamptics than the mild cases (Rahman & Ahmed 2015). The explanation given by Asnafi et al for the difference in the result between their study and other works is the difference in gestational ages at recruitment. In this study the gestational age of cases (34.67 ± 4.51 weeks) and controls (35.95 ± 7.54 weeks) at recruitment/diagnosis overlapped within the standard deviation with the studies by Rahman and Ahmed and Anim-Nyame and colleagues. The factor that stands between the participants in these studies and the current one is race. The account here confirms a similar study in South Africa in 2002 in which no significant difference was observed between the leptin levels in preeclampsia and pregnant controls (Kafulafula et al. 2002).
Leptin also showed no significant correlation with both diastolic and systolic blood pressure in preeclampsia and the pregnant controls. There was also no association between leptin and diastolic and systolic blood pressures in severe and mild preeclamptic subjects. The study found to report a significant correlation between blood pressure and leptin level was done in non-pregnant women (Khokhar et al. 2010). Acute increases in leptin has been found to have no effect on blood pressure because leptin in such concentrations stimulate both the vasoconstrictors (sympathetic nervous system) and vasodilators (nitric oxide dependent vasodilation and natriuresis) (Beltowski et al. 2010; Khokhar et al. 2010). However, chronic increase in leptin offset the balance by stimulating the vasoconstrictors only resulting in an increase in blood pressure. The leptin level of preeclamptics in this study was not different from the leptin level in the controls. Therefore, the vasoconstrictor effect that caused hypertension in the preeclamptics was not due to the influence of leptin, hence the absence of the correlation of leptin and blood pressure.

There was no difference in the gestational ages at which the preeclamptics and the pregnant controls were booked. The gestational age at diagnosis of severe preeclampsia was found significantly lesser than that of mild preeclampsia. This could be due to the lack of treatment of the preeclampsia that causes the early onset of the disease to develop in the severe form. Delivery is the only known cure to preeclampsia hence an increase in complication calls for the caesarean section on the mother to deliver the placenta and provide relief. This is the cause of the much lower gestational age at delivery in the preeclamptics than the controls. The uteroplacental under perfusion which restrict the amount of nutrients reaching the fetus in preeclampsia is the major contributor to the lower birth weight in preeclamptic babies than in babies of normal pregnancy with comparable gestational ages at delivery. Xiong found in 2002 a significant lower
birth weight in preeclamptic babies than in normotensive babies at $\leq 37$ weeks gestational age of delivery. However, a study by Xiong and Fraser in 2004 found no significant difference in the birth weight between preeclampsics and normotensives. With severity of the condition the birth weight decreases with lower gestational age at delivery.

5.4 LEPTIN AND PREECLAMPSIA SEVERITY

There was no significant correlation between leptin and blood pressure in all the situations considered, thus; normotensive pregnant, preeclampsia, mild preeclampsia and severe preeclampsia. The marker used here as a measure of severity is the blood pressure as used in other studies (Rahman & Ahmed 2015; Anim-Nyame et al. 2000). These past studies (Anim-Nyame et al and Rahman & Ahmed) measured serum free leptin as was carried out in this work. The difference in result is therefore probably due to the race of the study subjects as this work confirms another study in South Africa (Kafufula et al. 2002). Another possibility is the level of leptin binding protein in the blood. Higher levels of it affect the level of free leptin in the blood. The expression of the binding proteins like any other protein is affected by genetics which lead back to race being the peculiar factor here.

5.5 SUMMARY OF KEY FINDINGS

I. This study found no significant difference in leptin level between preeclamptic subjects and pregnant controls and between mild and severe preeclamptics at the Korle Bu Teaching Hospital.
5.6 LIMITATIONS OF THE STUDY

I. The study measured the free leptin level not the total leptin level in the blood which makes it difficult to actually understand whether or not leptin plays a role in preeclampsia because the level of free leptin is influenced by the level of leptin binding protein.

II. Pregnancy goes through three trimesters with great physiological changes. A longitudinal study of leptin level through the trimesters could have provided a better picture than the case control study.

5.7 CONCLUSION

The study found no significant difference in the circulating levels of leptin between the preeclamptics and normotensive subjects. Also, there was no significant difference in the leptin level between the severe and mild forms of preeclampsia in the study population. It therefore seems leptin plays no role in the pathogenesis and severity of preeclampsia.

5.8 RECOMMENDATIONS

I. A longitudinal study should be carried out to provide a profile of leptin dynamics of the three trimesters in preeclamptic and normotensive Ghanaian pregnant women.

II. Future studies should endeavor to concomitantly quantify free leptin and total leptin levels in preeclamptic subjects and pregnant controls to clear the dilemma of difference in leptin levels from data collated in Africa and that from other parts of the world.
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http://doi.org/10.1016/j.cca.2012.12.007
APPENDIX A

CONSENT FORM

Title: An evaluation of serum leptin dynamics in Preeclampsia at the Korle-Bu Teaching Hospital

Investigator: Emmanuel Abindau

Address: Department of Physiology, SBAHS, Box KB783, Korle-Bu

Information: (To be read or translated to patients in their own mother tongue)

Dear Volunteer

This consent form contains information about the research entitled An evaluation of serum leptin dynamics in Preeclampsia at the Korle-Bu Teaching Hospital. In order to be sure that you are informed about being in this research, we are asking you to read (or have read to you) this Consent Form. You will also be asked to sign it (or make your mark in front of a witness). We will give you a copy of this form. This consent form might contain some words that are unfamiliar to you. Please ask us to explain anything you may not understand.

Why this study is planned

You are being asked to participate in the above study in order to find out factors in the blood that may be of risk to the development of preeclampsia.

Pre Eclampsia is an illness of the pregnant seen in the form of high blood pressure (hypertension), leakage of protein into the urine (proteinuria), thinning of the blood (coagulopathy) and liver dysfunction. The disease is usually seen in the second trimester of the pregnancy or few days after delivery and has negative effect on the mother and unborn baby
during the pregnancy period and in later life both mother and child. Occasionally, Pre-clampsia can lead to convulsions (fits), a serious complication known as eclampsia. Also, when a pregnancy is complicated by preeclampsia, the baby may grow more slowly than normal in the womb or suffer a potentially harmful oxygen deficiency.

Preeclampsia is a very serious health problem in Ghana, as it is in many African countries and the cause of the disease is not yet known. Complications occur from this condition and sometimes lead to loss of life of mother, child or both. To understand this problem we need to study our pregnant women who come to the hospital with preeclampsia and compare them to normal pregnant women without preeclampsia, and to other normal non-pregnant women. The purpose of the study is to find out whether increased or decreased levels of leptin is associated with development of preeclampsia and its severity. If we can find the answer to this question, we hope to be able to suggest new and more effective preventive and management strategies for preeclampsia and its complications.

**General Information and your part in the study**

For you to qualify to be part of this study you should be between the ages of 16 and 45 years. If you agree to be in the study, we will collect venous blood sample for laboratory investigation. About 5ml of your venous blood will be sampled for the research at the time of admission. You will be asked some questions about yourself and your weight, height urine sample and blood pressure will be taken too. We will test the urine for protein.

**Possible Benefits**

There are no direct benefits to you from this study. However, your participation may help us develop new and more effective preventive measures as well as better treatment modalities for the management of preeclampsia. The high rate of maternal and perinatal morbidity and
mortality in Ghana and other African countries associated with preeclampsia will be reduced drastically if effective interventions are discovered as a result of this study.

**Possible Risks**

The amount of blood collected is harmless, although there may be slight pain and bruising at the bleeding site. All subjects will receive appropriate treatment as necessary. Sterile techniques and disposable, single-use equipment will be used at all times.

**Withdrawal from study**

We would like to stress that this study is strictly voluntary. Should you decide not to participate in the study it will have no consequences for you. Should the volunteer, at any point during the study, decide that she does not wish to participate any further, she is free to terminate the participation, effective immediately. Any such decision will be respected without any further discussion. Your decision will not affect the health care you would normally receive.

**Confidentiality**

All information gathered would be treated in strict confidentiality. We will protect information about you taking part in this research to the best of our ability. You will not be named in any reports. If you have any questions, please feel free to ask the physician in charge.

**Contacts:** If you ever have any questions about the research study or study-related problems, you may contact ; Emmanuel Abindau Department of Physiology, University of Ghana Medical School, (Tel 05408 74584), Dr. Kwame Adu-Bonsaffoh, Department of Obstetrics and Gynecology, University of Ghana Medical School, (Tel 0244 295 763) or Dr Daniel A. Antwi, Department of Physiology (Tel 0244 748 703) at any time. For questions about the ethical aspects of this study or your rights as a volunteer, you may contact the Chairman of the University of Ghana Medical School Ethical Review Board.
Your rights as a participant

This research has been reviewed and approved by the University of Ghana Medical School Ethical Review Board. An Ethical Review Board or Ethical Committee is a committee that reviews research studies in order to help protect participants. If you have any questions about your rights as a research participant, you may contact the Chairman of University of Ghana Medical School Ethical Review Board.

VOLUNTEER AGREEMENT

The above document describing the benefits, risks and procedures for the research titled “An evaluation of serum leptin dynamics in Preeclampsia at the Korle-Bu Teaching Hospital”, has been read and explained to me. I have been given an opportunity to have any questions about the research answered to my satisfaction. I agree to participate as a volunteer.

Date                                  Signature or Thumbprint of volunteer

If volunteer cannot read the form themselves, a witness must sign here:

I was present while the benefits, risks and procedures were read to the volunteer. All questions were answered and the volunteer has agreed to take part in the research.

Date                                  Signature or Thumbprint of witness
I certify that the nature and purpose, the potential benefits, and possible risks associated with participating in this research have been explained to the above individual.

------------------------------------------------                             -----------------------------------------------

Date                                                                          Signature Person who obtained Consent

Consent for controls

Title: The role of endothelial dysfunction in the pathogenesis of preeclampsia

Principal Investigator: Emmanuel Abindau

Address: Department of Physiology, SBAHS, Box KB783, Korle-Bu

Information: (To be read or translated to parents/guardians in their own mother tongue)
APPENDIX B

QUESTIONNAIRE
An evaluation of serum leptin dynamics in Preeclampsia at the Korle-Bu Teaching Hospital
INVESTIGATOR: EMMANUEL ABINDAU

PERSONAL DETAILS

| Name: | ………………………………………………… |
| Hosp. No: | ………………………………………………… |
| Age: | ………………… |

Ethnicity:

Contact: ………………………………..

Ethnic region of patient’s father

Ethnic region of mother

Educational Background: Primary JHS SSS Tertiary

Religion: No Religion Tradition Christian Muslim Other

Occupation: ………………………………..

Marital status: Single Married

Gravidity ……………… Parity: …………… Abortion …………………

Change of partner yes no

Does patient smoke yes no

Does patient drink alcohol yes no

Did patient get preeclampsia in previous pregnancies yes no

Does patient has a close relative who has ever had preeclampsia yes no

Maternal Outcome Indicators

Height (M) ………………………………..

Weight at booking (Kg) ………………………………..
BMI at booking (Kg/m²)…………………
Gestational age at booking………………
Blood Pressure at Booking………………
Gestational age at diagnosis……………
Blood Pressure at diagnosis………………
Urine protein at diagnosis………………
Birth weight……………………………..
Placenta weight………………………….
Gestational age at delivery………………

Biochemical Investigations
FBC (platelet count)……………………
BUE creatinine…………………………
[Uric acid]……………………………..
AST, ALT……………………………
Urine creatinine / protein ratio……………
Leptin……………………………………