MATERNAL SERUM HUMAN CHORIONIC GONADOTROPIN AND MAGNESIUM AS BIOCHEMICAL MARKERS IN PREDICTING PREECLAMPSIA IN PREGNANT GHANAIAN WOMEN.

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DECLARATION

I, Mohammed Mustapha Seini, declare that this project was duly undertaken by me at the Obstetrics and Gynecology Department of the Ridge Regional Hospital, Accra and at the Department of Medical Biochemistry, University of Ghana Medical School, Korle-Bu under close supervision of my supervisors.

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DEDICATION

I dedicate this work to my friends, who stimulated me and challenge me; to my family, who supported me; to my wife and child, Rukaya and Mickdad, who bring me happiness; and to my late Brother, Mohammed Shaibu Seini.

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ABSTRACT

Preeclampsia is a multisystem disease of pregnancy of unknown cause. It is a maternal syndrome, which is characterized by increased blood pressure, edema, proteinuria or significant amount of protein in the urine of pregnant women and abnormal clotting, liver and renal functions all of which may be due to the release of placental toxic factors into the mother's circulation. The only effective therapy to this complication is to facilitate the culteinment of the pregnancy or delivery (induction of labour, cesarean section). Biochemical markers such as human chorionic gonadotropin(hCG) and magnesium(Mg^{2+}) are used in predicting preeclampsia. No work has been done using both markers together and there is no maternal screening program for preeclampsia available for pregnant women in Ghana. The aim of this study was to determine the levels of maternal serum hCG and Mg^{2+} among cases and controls of pregnant Ghanaian women, with the view of assessing the differential if any in these markers, for high risk preeclamptic pregnancies. In this prospective study, 200 women aged between 16 and 40 years with singleton pregnancy were enrolled. Venous blood sample was collected at recruitment (second trimester) for Mg^{2+} and hCG assays. Levels of hCG were detected by ELISA methods, using commercial test kits and that of the Mg^{2+} were detected by the use of Selectral Junior analyzer also using commercial test kits. The study population was made up of 150 pregnant women in second trimester with features of preeclampsia and 50 controls (second trimester pregnant women with no features of preeclampsia). Out of the total women with features of preeclampsia, 13 pregnant women were diagnosed as preeclamptic patients with urine protein assigned+3.The mean age of the WFP was (29.65 ± 5.24) and that of the controls was (27.76 + 5.22). The serum markers, Mg^{2+} was significantly lowered (0.68 ± 0.11) whiles hCG significantly raised (0.99 ± 0.20) in the WFP. The study showed that hCG and Mg^{2+}

when used together provide a very good prediction of preeclampsia. Though Mg^{2+} is a better predictor of preeclampsia when used separately as compared to hCG

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ABBREVIATIONS

ANC	Antenatal care
BMI	-Body Mass Index
BP	-Blood pressure
95% CI	-95% Confidence interval
Circ	-Circumference
Cm	-Centimeter
Da	-Dalton
DBP	-Diastolic blood pressure
EDTA	-Ethylene Diamine Tetraacetic Acid
ELISA	-Enzyme Link Immuno- Sorbent Assay
F	-Female
Free β-hCG	-Free beta- Human Chorionic Gonadotropin
g/dl	-Gram per deciliter
Hb	-Hemoglobin
hCG	-Human Chorionic Gonadotropin
HIV	-Human Immunodeficiency Virus
IU/ml	-International Units per milliliter
Kg	-Kilogram
Kg/m^2	-Kilogram per meter square
LMP	-Last menstrual period
Ltd	-Limited

M	-Male
Mg^{2+}	Magnesium
M	-Meter
Max	-Maximum
Min	-Minimum
Ml	-Milliliter
mmHg	-Millimeters of Mercury
MoM	-Multiple of the median
N H LBI	National Heart, Lungs and Blood pressure Institude
N	-Number
ng/ml	-Nanogram per milliliter
Nm	-Nanometer
P	-P- value
PIH	Pregnancy Induced Hypertension
%	-Percentage
R	-Person's correlation coefficient
r^2	-Coefficient of determination
SBP	-Systolic blood pressure
SD	-Standard deviation
SGA	-Small for gestational age
UK	-United Kingdom
USA	-United States of America
USI	-Ultrasound investigation

UP	-Urine Protein
WFP	-Women with Features of Preeclampsia
WNFP	Women with No Features of Preeclampsia
WHO	World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

1.1 General introduction

Preeclampsia is a multisystem disease of pregnancy of unknown cause (Jaing *et al.,* 2002). It is a maternal syndrome, which is characterized by increased blood pressure(BP), tissue edema, proteinuria or significant amount of protein in the urine of pregnant women, abnormal clotting, abnormal liver and renal functions all of which may be due to the release of placental toxic factors into the mother's circulation(Janakiraman *et al.,2009*). The only effective therapy to this complication is to facilitate the curtailment of the pregnancy or delivery of the placenta (induction of labour, cesarean section). It appears likely that there are substances from the placenta that can cause endothelial dysfunction in the maternal blood vessel of susceptible women (Cunnigham *et al.*, 2001).

Preeclampsia has been shown to be associated with pregnancy induced hypertension (PIH) also known as gestational hypertension or transient hypertension in pregnancy. It is characterized by the development of BP of greater than 140/90mm Hg after 20 weeks of gestation (repeatedly for four to six hours apart)(Munjuluri *et al*.,2005). Preeclampsia is diagnosed when additionally the pregnant woman develops new onset of significant proteinuria of more than 300mg /liter or greater than 300mg/ 24 hr urine collections after 20 weeks of gestation. This usually occurs over several days to weeks, but may occur more quickly (Papageorghiou *et al.*, 2001)

The following maternal signs and features characterize severe preeclampsia (Douglas and Redman,1994).

1. Blood pressure greater than 160/110 mm of Hg(on two occasions four to six hours apart)
2. Severe headache
3. Epigastric pain
4. Cerebral and visual disturbance (blurring, scotomata, flashes of light)
5. Proteinuria greater than 5mg/24hr or more than +1
6. Pulmonary edema
7. Oliguria(urine output less than 500ml/ 24hr or 20ml / hr)
8. Low platelet count(Thrombocytopenia)
9. Liver enzyme abnormalities.
10. Elevated serum creatinine and
11. Brisk deep tendon reflexes (hyper reflexia)

Pre-eclampsia involves generalized damage to the maternal endothelium, kidneys and the liver. Apart from caesarean section or induction of labour and therefore delivery of the placenta, there is no cure (Skjaerven *et al.*, 2002). Biochemical markers such as human chorionic gonadotropin (hCG) and magnesium (Mg^{2+}) are used in predicting preeclampsia.

The purpose of hCG, which is produced by the trophoblast is to maintain an important temporary maternal endocrine cell group called the corpus luteum which continues to produce or secrete oestrogen and progesterone until 8-9 weeks of pregnancy (Hibbard and Rosen,1977). Thereafter the placenta becomes the main source of oestrogen and progesterone. When a woman ovulates and releases an egg, the cells that surrounded the egg while it was in the ovary become hormone-secreting cells collectively called the corpus luteum. If an egg is not fertilized, its corpus luteum dies within about 14 days (Rumbold *et al.*, 2006). In the case of fertilization, hCG maintains the

corpus luteum. hCG also maintains the lining of the uterus, which is necessary to sustain a pregnancy if an egg is fertilized. By maintaining the uterine lining, hCG provides a conducive place for embryonic development (Cole, 1999).

Although hCG primary role in pregnancy relates to the developing embryo, it also has a separate utility. Physicians can test for human chorionic gonadotropin levels in blood to monitor the viability of an early pregnancy. The earliest form of human chorionic gonadotropin expressed by trophoblasts seems to facilitate embryo implantation, human chorionic gonadotropin also stimulate ovarian progesterone secretion in quantities sufficient for the maintenance of pregnancy (Gokdeniz *et al*,,1999).

The importance of Mg^{2+} in pregnancy has been elucidated and emphasized by several studies (Lopez *et al*,. 2004). Magnesium is considered a membrane-stabilizing ion and a permeability regulator acting at an intracellular level which intervenes in the oxidative phosphorylation and oxidation reduction processes as well as in enzymatic synthesis and activity (Paolisso *et al*,. 2006). A deficiency in magnesium could possibly lead to spasms in the placenta and the umbilical cord. These spasms could lead to premature labor and increases the risks of birth defects or even infant mortality (Peacock, 1999). Magnesium helps to relax the muscles, and getting the optimal amount of magnesium has been shown to relax the uterus thereby allowing the pregnancy to continue for its normal duration, Magnesium is also used by most biosynthetic enzymes such as DNA polymerase and RNA polymerase as cofactors (Dahle *et al.*, 2003).

1.2 Research problem

Many women in Ghana suffer the effects of pre-eclampsia during pregnancy and this may lead to maternal mortalities. The diagnosis and early management of preeclampsia poses a challenge to obstetricians and gynaecologists. The incidences of preeclampsia (7.03 %) contribute to the morbidity and mortality rate in Ghana (Obed and Aniteye, 2006).

Between 12 and 15 pregnant women are diagnosed with preeclampsia in Ridge Hospital quarterly. Eighty percent (80%) of these women undergo caesarean section with thirty nine percent (39%) fetal mortality (Ridge Hospital records, 2011).

1.3 AIM:

The aim of this study was to investigate the use of serum hCG and Mg^{2+} as two predictive biochemical markers for predicting pre-eclampsia in pregnant Ghanaian women.

1.4 OBJECTIVES

The objective of this study was to;

i. Determine the serum values of hCG and Mg^{2+} in pregnant Ghanaian women in the second trimester of gestation.

ii. Determine if serum values of the hCG and Mg^{2+} could predict pre-eclampsia in the pregnant Ghanaian women.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Pathogenesis of preeclampsia

The causes of preeclampsia remain unknown. Therefore, an attempt to define pathophysiological data in one causal framework represents another one of the many hypotheses proposed to explain the pathogenesis of preeclampsia (Fisher, 2004).

Preeclampsia is caused by presence of the placenta or the maternal response to placentation. Preeclampsia is characterized by abnormal vascular response to placentation that is associated with increased systemic vascular resistance, enhanced platelet aggregation, activation of the coagulation system, and endothelial-cell dysfunction (Koelman *et al.*, 2000). Several studies have suggested that women who develop preeclampsia are at increased risk of cardiovascular complications later in life (Jelto, 2005). Indeed, many risk factors and pathophysiological abnormalities of preeclampsia are similar to those of coronary-artery disease (Acromite *et al.*, 1953).

The cause of preeclampsia is often described by two opposing schools of thoughts—the vascularists, for whom ischaemia-reperfusion leads to oxidative stress and vascular disease, and the immunologists, who see preeclampsia as a maternal–paternal immune maladaptation (ie, a maternal alloimmune reaction triggered by a rejection of the fetal allograft) (Dekker,2002).

Pre-eclampsia occurs mainly in first pregnancies. This has been explained by the invoking of immune mechanisms and is linked to the belief that a genetically foreign fetus challenges the maternal immune system. The hypothesis is that the maternal immune system ‘learns’ to

accommodate the fetus. Such adaptation may be relatively defective in a first pregnancy but less so in subsequent pregnancies (Do *et al*., 2002). Furthermore, there may be partner specificity, which strengthens the argument that pre-eclampsia results from a relative failure to induce maternal tolerance of paternal smooth muscle, coagulation, and other immune cells that are most relevant to preeclampsia (Cunningham *et al.*, 2001).

The following theories have been described to explain the pathogenesis of preeclampsia.

2.1.1 Placentation and the immune theory of preeclampsia

This theory includes abnormal placentation, placental oxidative stress and immunological factors that can be summarized as follows:

2.1.1.1. Impaired trophoblast invasion and differentiation

In human pregnancy implantation of the embryo occurs by two physiological invasions of the cytotrophoblast inside the uterine wall. First, cytotrophoblast cells stream out of the tips of the anchoring villi and penetrate the trophoblast shell and overlying syncytiotrophoblast to form cytotrophoblast columns that develop into the cytotrophoblast shell. Trophoblast cells continue to migrate into the decidua. After an apparent long pause (6–8 weeks) at the beginning of second trimester (14–16th week) of gestation, a second very deep trophoblast invasion colonizes the placental bed's myometrium (Skjaerven *et al.*, 2002). Once the cytotrophoblast makes contact with spiral-artery openings, trophoblast cells stream into the arterial lumina to form intraluminal plugs. Endovascular trophoblast cells replace the endothelium of spiral arteries and then invade the media, resulting in destruction of the medial elastic, muscular, and neural tissue. Trophoblast cells become incorporated into the vessel wall, and the endothelial lining is finally reconstituted. As a result, these vessels undergo transformation from small muscular arterioles to large capacitance vessels of low resistance (Laresgoiti *et al*., 2010).

Trophoblast differentiation during spiral arteries invasion involves alteration in expression of the number of different classes of molecules, including cytokines, adhesion molecules, extracellular matrix molecules, metalloproteinases, and the class Ib major histocompatibility complex molecule, HLA-G. During normal differentiation, invading trophoblasts alter their adhesion molecule expression from those that are characteristic of epithelial cells to those of endothelial cells, a process referred to as pseudo-vasculogenesis (Skjaerven *et al.,* 2002).

The proper trophoblast invasion and switching in the adhesion molecules allows increased blood flow (i.e., oxygen, nutrients) to the fetus. It has been proposed that the trophoblasts obtained from women with preeclampsia do not show upregulated adhesion molecules expression or pseudo-vasculogenesis (Skjaerven *et al.,* 2002). The impairment of pseudo-vasculogenesis limits the cytotrophoblast infiltration only to the decidual portion of the spiral arteries and fails to penetrate the myometrial portion and inadequate transformation of spiral arteries around the 14–16th week of the gestation, inducing poor vascular exchanges between the mother and the placenta. The rise of blood pressure in the pregnant woman is then probably a compensatory mechanism to increase the exchanges and try to save the fetus from poor supplies. Whether this adaptation is maternally driven or a response to fetal stress signals is yet unknown (Skjaerven *et al.,* 2002).

2.1.1.2 Graft rejection of the feto–maternal allograft

Since half of the fetal genome is derived from the father, the fetus synthesizes antigens considered to be foreign by the maternal immune system therefore, mother should reject it, but this generally does not occur. In his classic paper, Acromite *et al*.,1953, proposed the concept of the "fetal allograft" to explain the immune relationship between mother and fetus for successful pregnancy out-come.

In this model, three hypotheses were proposed by the authors

1) That the conceptus lacked immunogenicity

2) That there was a significant lowering of immune response during pregnancy

3) And that there is the elaboration of an immune barrier by the placenta. Through the years, the third hypotheses, suggesting the existence of an immune barrier elaborated by the placenta, have acquired considerable attention. Originally, this barrier was presumed to be passive or neutral but later the placenta was shown to be a site of active tolerance. It is presumed that fetal cells and molecules are released into the maternal blood during proliferation of trophoblastic cells, following tissue ruptures that occur at the terminal extremity of the growing chorionic villi. The whole immune system comes into contact with these potential fetal immunogens. Therefore, tolerance to the semi-allogenic fetus by the maternal immune system seems mainly an active mechanism whereby fetal tissues are prevented from being recognized as foreign (Acromite *et al*., 1953).

2.1.1.3 Trophoblast development

Taking a look at early human development the trophoblast is the first cell lineage to differentiate at the stage of the blastocyst at about day 6 postconception (p.c.). Further differentiation steps result in the formation of the 2 different pathways of trophoblast, the villous and the extravillous pathway. Preeclampsia is the result of a failure of villous trophoblast differentiation, which on the placental side ultimately leads to an abnormal release of trophoblast material into the maternal circulation as shown below (Fig 1).

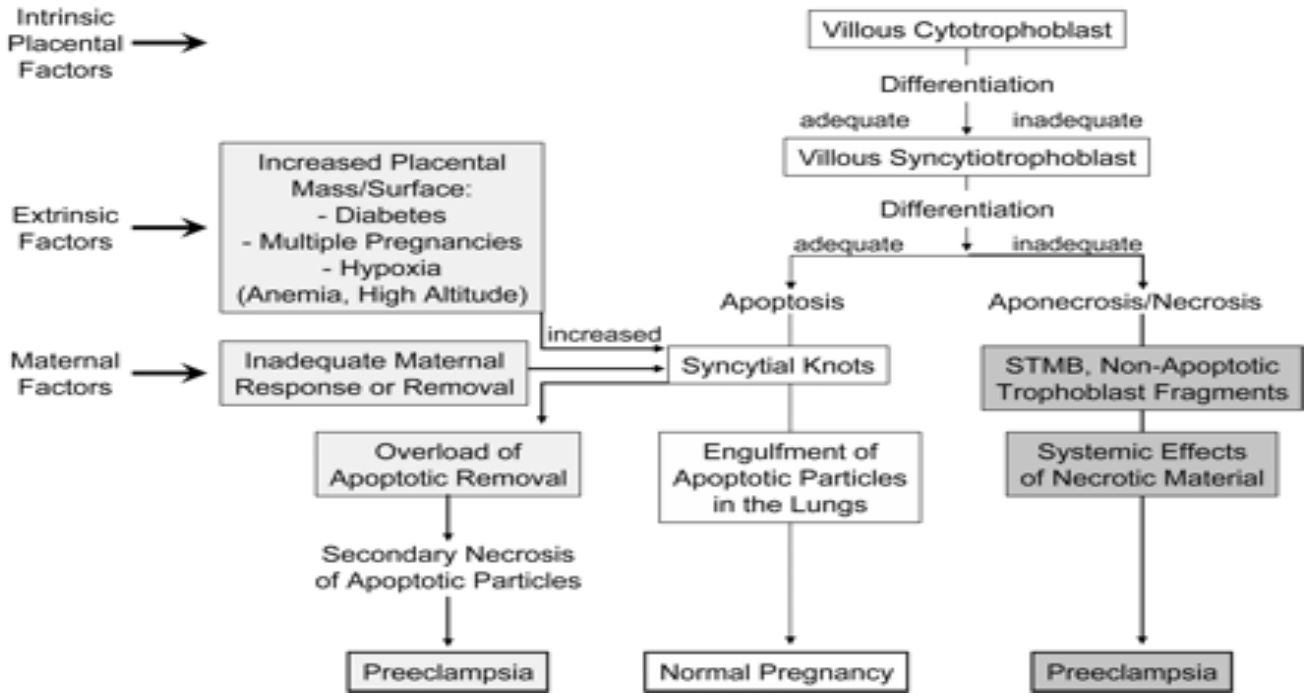


Fig 1. Normal and adverse pregnancy pathways (Fisher, 2004).

Fig 1 represents an overview of the events occurring during normal pregnancy and leading to the development of preeclampsia. The normal pathway (in light gray) starts with normal cytotrophoblast differentiation and ends with the engulfment of apoptotic syncytial knots in the lungs. If preeclampsia is initiated by intrinsic placental factors there is a shift toward nonapoptotic release of trophoblastic fragments resulting in preeclampsia (in dark gray). Due to extrinsic factors the maternal disposal system to remove apoptotic syncytial knots may be overloaded resulting in secondary necrosis of syncytial knots and subsequently in preeclampsia (in gray). It has also been shown that due to other maternal factors the mother may not respond adequately to the presence of placental material in maternal blood (in gray) and may have the same outcome as the presence of extrinsic factors: preeclampsia (Fisher, 2004).

2.1.1.4 Normal pregnancy

During normal pregnancy aged and late apoptotic syncytiotrophoblast nuclei are packed into apical protrusions of the syncytiotrophoblast called syncytial knots (Fig1, in light gray). These membrane-sealed corpuscular structures are apoptotically generated and released from the apical syncytiotrophoblast membrane into the maternal circulation. They are transported through the maternal venous system behind the placenta and reach the first capillary bed behind the placenta, the lungs. Here these huge apoptotic structures, containing multiple nuclei, are engulfed by lung macrophages (Fig 1, in light gray) and thus cannot be detected in peripheral maternal blood behind the lungs. In the peripheral blood of healthy pregnant woman these structures are virtually absent. It has been described that the engulfment of apoptotic material by macrophages leads to a silencing of such macrophages, thus reducing the secretion of proinflammatory cytokines (Fisher, 2004).

2.1.1.5 Intrinsic Placental Factor - Induced preeclampsia

During preeclampsia the release of the syncytiotrophoblast material does not follow the normal pathway as described above. Due to an alteration in villous trophoblast differentiation early in pregnancy, the release of syncytial knots is not more the main mechanism of disposal. Now other mechanisms such as necrosis and aponecrosis take over (Fig 1, in dark gray). The latter term describes the start of the apoptosis cascade followed by a failure of the program to end normally. This then results in a necrotic release of already apoptotically cleaved material. The two mechanisms, necrosis and aponecrosis, give rise to the necrotic and cell-free release of trophoblast material. Such necrotic trophoblast fragments can be detected in high numbers only in preeclampsia whereas in fetal growth restrictions they are not elevated above normal levels (Fisher, 2004).

Although early serum markers may predict preeclampsia already in the first trimester of pregnancy, the respective clinical manifestation of preeclampsia only occurs after mid-gestation. This discrepancy can be explained as follows: during the first trimester of pregnancy trophoblast turnover is different to the turnover later in gestation. In the first trimester most of the fusion events of cytotrophoblast cells with the syncytiotrophoblast are needed for growth of the syncytiotrophoblast rather than for the maintenance of this layer. Only later in gestation does a steady-state between input of new material by cytotrophoblast fusion and release of syncytial knots is established. During the first trimester the release of trophoblast material is much lower than later in gestation. This is not only true because of the lower total placental mass and surface early in gestation but also because of the differences in trophoblast turnover at the two different stages of trophoblast development (Fisher, 2004).

2.1.1.6 Extrinsic and Maternal Factors - Induced preeclampsia

The origin of preeclampsia may not be restricted to an intrinsic alteration of the villous trophoblast alone. Specific conditions increase placental mass (diabetes or multiple pregnancies) or placental surface hypoxic conditions of the mother: anemia, high altitude (Fig 1, in gray pathway on the left depicts extrinsic factors). This increase will be paralleled by an increase in the release of syncytial knots. If the maternal clearance system cannot cope with this increased number of apoptotic fragments, they may undergo secondary necrosis within the blood with altered endothelial damage and thus may lead to the clinical symptoms of preeclampsia as well (Fisher, 2004).

The same may be true if the maternal disposal or inflammatory systems are not working properly and react inappropriately to the release of apoptotic trophoblast fragments (Fig 1,in gray pathway on the left, maternal factors). Again, this may lead to an overload of the disposal

machinery, thus inducing a systemic activation and damage of endothelial cells, resulting in preeclampsia (Fisher, 2004).

2.2 Risk factors for the development of preeclampsia

Various risk factors have been proposed in the pathogenesis of Preeclampsia. These include;

1. History of preeclampsia, a family history of preeclampsia increases the risk of developing the condition (20-25%). It is most probably inherited as a single recessive maternal autosomal gene (Obrien, 1993).
2. First pregnancy, the risk of developing preeclampsia is highest during first pregnancy or first pregnancy with a new partner (primipaternity) (Obrien, 1993)
3. Age, the risk of preeclampsia is higher for pregnant women younger than 16 years and older than 40years (extreme of reproductive age) (Escen *et al.*, 2003)
4. Previous Eclamptic women have about 25-60% risk of developing preeclampsia (Neal *et al.*, 2004)
5. Obesity, The risk of preeclampsia is higher with heavy pre-pregnancy body weight (Bonzini *et al.*, 2007).
6. Multiple pregnancy, Preeclampsia is more common and more serious with women who are carrying twins, triplets or higher multiples (2x) (Long and Oast, 1987).
7. Donor egg, the incidence of preeclampsia is higher in women who become pregnant with donor egg (Salha *et al.*,1999).
8. Prolonged interval between pregnancies, this seems to increase the risk of preeclampsia (2-3x) (Skjaerven *et al.*, 2002).

9. Gestational diabetes, women who develop gestational diabetes have a higher risk of developing preeclampsia as the pregnancy progresses (Pool *et al.*, 2010).
10. History of medical conditions, having certain conditions before pregnancy — such as chronic high blood pressure, migraine headaches, diabetes, kidney disease, rheumatoid arthritis or antiphospholipid syndrome and anti cardiolipin increases the risk of preeclampsia (Altura *et al.*, 1994).

Other factors that may be associated with a higher risk of preeclampsia include:

Having other health conditions, both urinary tract infections and periodontal disease during pregnancy are associated with an increased risk of preeclampsia, which may indicate that antibiotics could play a role in prevention of preeclampsia (Pipkin *et al.*, 2001).

Vitamin D insufficiency, insufficient vitamin D intake increases the risk of preeclampsia, and that vitamin D supplements in early pregnancy could play a role in prevention. (Pipkin *et al.*, 2001).

Furthermore high levels of glycoprotein such as hCG in the serum of pregnant women have been found to be more likely to develop preeclampsia than are other women. These proteins interfere with the growth and function of blood vessels lending evidence to the theory that preeclampsia is caused by abnormalities in the blood vessels feeding the placenta.

2.3 Complications of preeclampsia:

I, Preeclampsia affects the arteries carrying blood to the placenta. If the placenta does not get enough blood, due to the lack of blood flow to the placenta, the fetus may receive less oxygen and fewer nutrients. This can lead to slow growth, low birth weight, preterm birth and breathing difficulties (Pipkin *et al.*, 2001).

II, Preeclampsia increases the risk of placental abruption, in which normally situated placenta separates from the inner wall of the uterus before delivery. Severe abruption can cause heavy bleeding, which can be life-threatening for both the mother and the fetus (Pipkin *et al.*, 2001).

III, HELLP syndrome, HELLP which stands for Hemolysis, Elevated Liver enzymes and Low Platelet count syndrome can rapidly become life-threatening for both mother and fetus. Symptoms of HELLP syndrome include nausea and vomiting, headache, and upper right abdominal pain and jaundice. HELLP syndrome is particularly dangerous because it can occur before signs or symptoms of preeclampsia appear (Sibai *et al.*, 2003).

IV, Eclampsia, when severe preeclampsia is not controlled eclampsia which is essentially preeclampsia plus seizures can develop. Features of severe pre-eclampsia include upper right abdominal pain, severe headache, vision problems and change in mental status, such as decreased alertness. Eclampsia can permanently damage the vital organs, including the brain, liver and kidneys. If left untreated, eclampsia can cause coma, brain damage, Hepatic rupture, renal failure, cerebral hemorrhage, pulmonary oedema, cortical blindness and maternal death, cerebral edema, cerebro vascular accident and hypertensive encephalopathy (Khatun *et al.*, 1997),

V, Acute renal failure, acute tubular necrosis and bilateral cortical necrosis (Pipkin *et al.*, 2001).

2.4 Prediction of preeclampsia

The prevalence of preeclampsia in developing countries ranges from 1.8% to 16.7% (Canfield *et al*, 2006). Many challenges exist in the prediction, prevention, and management of preeclampsia. Promising prophylactic measures like low-dose aspirin and calcium supplementation need further evidence before recommendation for use in developing countries. Treatment of preeclampsia remains prenatal care, timely diagnosis, proper management, and timely delivery (Osungbade *et al.*, 2010)

The prevalence ranges of preeclampsia in some countries are shown in table 1.

Table1. **Global Percentage Prevalence ranges of preeclampsia**

Source	**Percentage ranges**	
	From	**To**
South Africa	**1.8**	**7.1**
Egypt	**1.8**	**7.1**
Tanzania	**1.8**	**7.1**
Ethiopia	**1.8**	**7.1**
Nigeria	**2.0**	**16.7**
North America	**0.05**	**0.07**
Europe	**0.05**	**0.07**

Source: WHO report, 2005.

As a result of the alarming prevalence of preeclampsia, a huge number of tests have been proposed to predict preeclampsia ranging from standard methods of antenatal care such as blood pressure measurements and proteinuria by dipstick to blood and urine biochemical tests, infusion of vasoconstrictor substances, haematological markers and ultrasonograph evaluation of placental vessels (Lam *et al., 2006).*

There is currently no single reliable, cost-effective screening test for preeclampsia. The serum uric acid level was once used as an indicator of preeclampsia but has been found to lack sensitivity and specificity as a diagnostic tool. However, an elevated serum uric acid level may be of some use in identifying pregnant women with chronic hypertension who have an increased likelihood of having superimposed preeclampsia (Magee *et al.*, 2003).

Due to the fact that none of these methods combines accuracy, reproducibility and simplicity to become a universal predictive marker of preeclampsia, there continues to be a compelling demand for new markers.

2.4.1 Human Chorionic Gonadotropin

2.4.1.2 hCG structure and synthesis

Human Chorionic Gonadotropin is a glycoprotein with a molecular weight of approximately 37,500 Da (Cole *et al*, 1991). Structurally intact hCG molecules consist of two non- covalently linked polypeptide, alpha and beta chain subunits. The alpha subunit, a 92- amino acid sequence, with a molecular weight of approximately 14,000 Da, is identical to that of luteinizing hormone, follicle-stimulating hormone, and thyroid - stimulating hormone (Saldana, *et al., 2009*).The beta subunit, a 145- amino acid sequence, with molecular weight of approximately 23,500 Da, is

unique to hCG. It confers biological and immunological specificity to the entire hCG molecule (Cole *et al.*, 1991).

The hCG is first produced by the syncytiotrophoblasts of the placenta after implantation of the embryo (usually 5-8 weeks after conception) and in later pregnancy by the chorion and placenta (Cole *et al.*, 1991).

Five hCG related molecules are present in maternal serum: nonnicked hCG, which represent an active hormone; nicked hCG, free alpha subunit, free beta subunit; and the nicked free beta subunit (Cole *et al.*, 1991).

Normal pregnancy is associated with an exponential increase of both holo-hCG and its free beta subunit. The serum concentration of hCG starts increasing 4-7 days after implantation. During early pregnancy, the hCG concentration increase exponentially doubling on average every 1.5-2 days, but the rate of increase varies individually and maximum concentrations ranging from 20000 to 100000 IU/L are reached at 7-10 weeks of pregnancy. After this, the levels gradually decrease during the second and third trimesters of pregnancy. After delivery, hCG returns to an undetectable level (Cole *et al.*, 1997).

The free β-hCG corresponds to only 0.3-4% of the total hCG levels (Reis *et al*., 2002). As in the total hCG the serum concentration of free β-hCG increases rapidly to reach maximum levels of 60 ng/ml at 8-9 weeks of pregnancy, followed by a gradual decline during the next 11-12 weeks of gestation (Brajenovic *et al.*, 2004).

2.4.1.3 Biological function of hCG

One physiological function of hCG in pregnancy is to maintain progesterone and estrogen production of the corpus luteum during the first six weeks of gestation (Stenman *et al.*, 2006) and testosterone synthesis by the testes in male fetuses (Welch and Malone, 2002). After six weeks of gestation the syncytiotrophoblast cells synthesize progesterone independent of hCG stimulation until term. A recently identified function of hCG is to maintain maternal blood, support hemochorial placentation and provide nutritional support for the fetus (Cole, 2010).

Free β-hCG lacks hCG activity, but several studies indicate that it exerts growth-promoting activity (Cole, 2010). Maternal hCG levels seem to be increased in the second trimester in pregnancies that subsequently develop pre-eclampsia.

There is general agreement that the placenta remains the main source of hCG in patients with preeclampsia. The hCG secretion may be affected as a result of abnormal placental invasion or placental immaturity. Compared with normal pregnancies, the placentae of patients with unexplained elevated maternal hCG levels tend to be larger and to have an increase density and intensity of hCG (Al-Sebai *et al.*, 1996). A pregnant woman with elevated hCG levels usually above 2.0 MoM between 16 and 34 weeks of gestation is suggestive of preeclampsia (Myatt and Miodov, 1999).

An elevation in serum hCG levels in the second and third trimesters has been linked to the development of preeclampsia and other adverse pregnancy outcomes. It can predict an increased risk of perinatal death, low birth weight, small-for-gestational-age infants, preterm premature rapture of membranes, and preterm birth (Myatt and Miodov, 1999). A correlation between

elevated serum free β-hCG levels and severe preeclampsia in third trimester has been reported (Myatt and Miodov, 1999).

2.4.2 Magnesium (Mg^{2+}):

Magnesium is the 4th most abundant cation in the body and is present in more than 300 enzymatic systems where it is crucial for ATP metabolism (Whang,1997). Magnesium may influence blood pressure by modulating vascular tone and structure through its effects on myriad biochemical reactions that control vascular contraction/dilation, growth/apoptosis, differentiation and inflammation. Magnesium acts as a calcium channel antagonist (Tucker *et al*,. 1999). It stimulates production of vasodilator prostacyclins and nitric oxide and alters vascular responses to vasoconstrictor agents. Its deficiency can also play a role in hypertension of pregnancy (Liao *et al.*, 1996).There are many studies supporting the hypothesis that low serum magnesium is a risk factor for developing hypomagnesaemia hypertension and vascular dysfunction, these findings also support the hypothesis that is one of possible etiologies of pre-eclampsia (Handwerker *et al.*, 1996).

2.4.2.1 Functions of Magnesium

Magnesium functions intracellularly as a necessary cofactor of greater than 300 enzyme systems, and a decrease in cellular Mg^{2+} would result in partial membrane depolarization and decreased repolarization in association with cellular calcium accumulation and potentiated calcium-dependent cell actions including, smooth muscle, vasoconstriction; in neural tissue, enhanced sympathetic activity; and in skeletal muscle and fat tissue, insulin resistance (Shechter *et al*., 2003). These alterations have indeed been reported in cellular Mg^{2+} deficient states, such as essential hypertension and noninsulin-dependent diabetes mellitus (Flowers, 1965). Furthermore,

these same defects can be induced experimentally by dietary Mg^{2+} depletion, directly causing vasoconstriction or vascular spasm in various vascular beds including cerebral, coronary, and placental vessels as well as elevated BP, increased neuromuscular irritability (Flowers, 1965).

Historically consistent with the above, it was the ability of Mg^{2+} to suppress neural irritability that first led investigators more than 70 years ago to use Mg^{2+} therapeutically in preeclamptic pregnancy and magnesium sulfate remains a standard therapeutic maneuver and the drug of choice to prevent convulsions in women with preeclampsia, although the exact mechanism of action of Mg^{2+} remains unknown (Altura *et al.*, 1994). Two recent randomized trials have documented that Mg^{2+} sulfate is superior to a placebo for prevention of convulsions in women with severe preeclampsia. Among all women enrolled in the large Magpie trial (2002), one of the largest randomized trials to date that enrolled ten thousand one hundred and forty one women with preeclampsia in 33 nations, the rate of eclampsia was significantly lower in those assigned to Mg sulfate (0.8% versus 1.9%; relative risk, 0.42; 95% confidence interval, 0.29, 0.60) (Ascherio *et al*., 1998). A recent Cochrane systematic review has shown that magnesium sulfate is superior to other regimens for preventing eclamptic seizures, more than halving the risk, and may reduce the risk of maternal death, although not improving the outcome for the baby. However, despite the long-standing therapeutic use of intravenous magnesium sulfate in preeclampsia, oral magnesium supplementation does not seem to influence the incidence of preeclampsia (Altura *et al.*, 1994).

Clinically, Mg^{2+} deficiency (<1.5 - 2.5 mg/dL or < 0.7 mmol/ml) and preeclampsia share many features, including placental vasospasm, elevated BP, and increased neuromuscular irritability. This and other previous findings of cellular Mg^{2+} deficiency in essential hypertension, made it

seem reasonable to investigate the possibility that endogenous-tissue Mg^{2+} depletion might underlie or predispose to at least some pathophysiological aspects of preeclampsia (Altura *et al.,* 1995).

The overall risk for using magnesium sulfate in a pregnant or laboring mother is not fully understood. It is noted that the drug relaxes muscle tissue and is thought to be useful in the prevention of preterm labor. For a mother who is full term, this could create problems by lengthening her labor and increasing her risks for interventions like cesarean section (potentiates the effects of non depolizing neuromuscular blockers in anesthesia) or forceps delivery. Tests have also shown that during treatment with magnesium for preeclampsia, the drug does cross the placenta in a large enough volume to reach the fetus. The effects and complications of this placental crossing could cause are not yet known (Escen *et al.,* 2003).

Magnesium sulphate has been used for treating eclampsia in the United States for much of the 20th century. The international collaborative Magpie eclampsia trial (2002) confirmed that this anticonvulsant is indeed more effective, and safer, than alternative drugs. British obstetric practice has changed rapidly in response to these findings, and standard treatment of eclampsia in the United Kingdom now much more closely corresponds to that of the United States, although some controversies remain about optimal dosage (Escen *et al.,* 2003).

There is now some evidence that, for women with pre-eclampsia, magnesium sulphate more than halves the risk of eclampsia and probably reduces the risk of maternal death (Magee *et al.*,2005). Magnesium sulphate is clearly the anticonvulsant of choice for treating eclampsia, with substantial reductions in the risk of further seizures compared with diazepam, phenytoin, and lytic cocktail. It is also better at preventing maternal death than diazepam. Compared with

phenytoin, magnesium sulphate has a lower risk of pneumonia and ventilation, as well as being safer for the fetus (Magee *et al.*, 2005). Maternal ages between 30-40 years are among the clinical risk factors for the development of preeclampsia. The increased incidence of preeclampsia noted among patients older than 35 years probably reflects undiagnosed chronic hypertension with superimposed PIH (Neal *et al.*, 2004).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1. Study Design and settings

3.1.1 Ethical consideration

The study was approved by the Research and Ethical Review Committee of the University of Ghana Medical School, Legon with protocol identification number MS-Et/M.6-P 3.2/2012-13.

3.1.2. Sample size determination

The minimum sample size for the study was determined using the formula (McClave and Dietrich, 1979):

$N= z^2 (p) (1-p) \times 2/ (error)^2$

Where N = sample size, Z = standard score for the confidence interval of 95% which equals 1.96. P is the sample proportion of the prevalence of the combined probability of a pregnant woman having pre-eclampsia at age 40 or less, which equals 7.03% (Obed and Aniteye, 2006). Assuming an error of 5% in this estimate, the sample size per a risk group for pre-eclampsia is 200.

3.1.3 Recruitment of study participants

A total of 250 pregnant women attending the antenatal clinic of the Department of Obstetrics and Gynecology, Ridge Regional Hospital, Accra, Ghana were interviewed over a period of 13 months (from 1 July, 2011 to 31st July, 2012). Out of these 200 singleton pregnant women were found eligible and recruited into the study (Fig 2). Detailed information was given about the

study and informed consent obtained for participation in the study. Each subject was randomly and consecutively selected, representing a cross- sectional study of the Ghanaian population.

The gestational age at recruitment was 14-24 weeks (second trimester). Gestational age was estimated from the date of last menstrual period (LMP), if it was available, and confirmed from the abdomen/pelvic ultra sound scan. The abdomen/pelvic ultra sound scan also looked for fetal defects and multiple pregnancies. Gestational age was expressed in completed weeks (eg 12 weeks 6 days, is taken as 12 weeks).

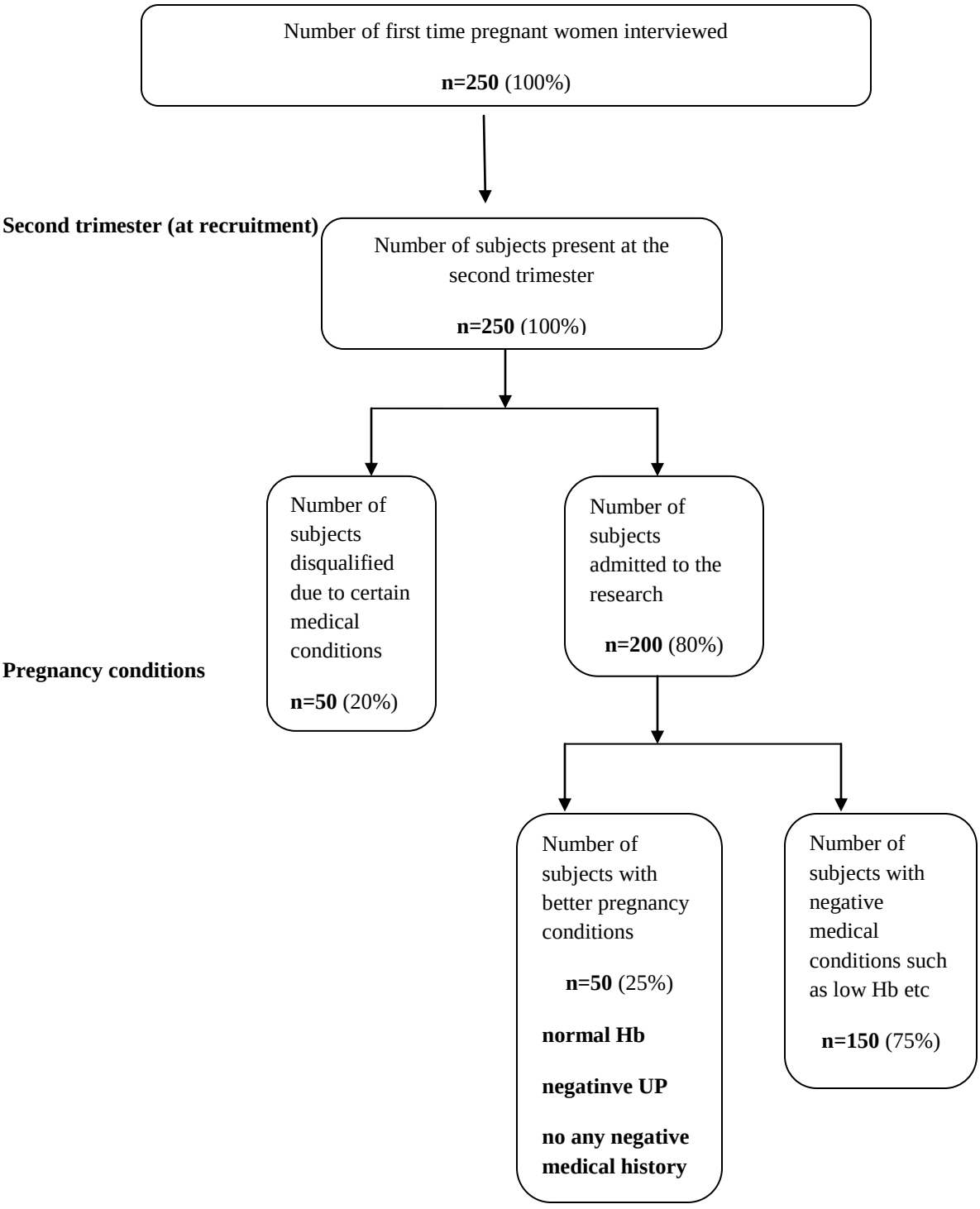
At the time of recruitment, each subject completed a questionnaire with the help of the principal investigator. The questionnaire obtained information on maternal age, maternal weight, and height, smoking habits, alcohol intake, intake of drug supplements (such as iron, folic acid and vitamins), marital status, religion, tribe, educational background, occupation, blood group and Rhesus factor, family history of high BP and gestational hypertension.

The weight was measured to the nearest kilograms (kg) and height to the nearest centimeter (cm) to calculate the Body Mass Index (BMI). Blood pressure was taken after the subjects had rested for 15 minutes.

Venous blood sample was taken for measurement of hemoglobin (Hb) and for Mg^{2+} and hCG assays at the second trimester. The recruitment gestational period (14-24 weeks) was chosen as a baseline to compare with the control data of the biochemical markers. The dip stick method of measuring urine protein was used to determine the protein levels in the urine of all the 200 women.

The information concerning pregnancy outcomes was obtained from medical records of the women.

Fig2. **Flow chart of study subjects' participation at recruitment**



3.1.4 Inclusion criteria

First time pregnant women, who were aged 16- 40 years with singleton pregnancy, were included in the study.

3.1.5 Exclusion criteria

First time pregnant women who were aged less than 16 years and more than 40 years or had multiple pregnancies were excluded from the study.

3.1.6 Blood sample collection and storage

After recruitment 5ml of maternal blood was obtained from each pregnant woman into serum separator vacutainer tubes for determination of hCG and Mg^{2+}. A portion was aliqouted into EDTA vacutainer tubes for measurement of hemoglobin.

The samples in the serum separator vacutainer tubes were allowed to clot for 30 minutes before centrifugation for 15 minutes at 10000 x g. The resulting serum was aliquoted into eppendorf tubes for storage at – 20^0C till analyzed.

3.2 Chemicals, reagents and kits

Human Chorionic Gonadotropin and Magnesium immunoassay kits were obtained from GenWay Biotech, Inc., San Diego, USA. With Ref numbers DNOV034 and DNOB019, batch numbers B-hCG 2917.and B-Mg 0023 and expiry date 31-12-2014 and 31-12-2014 respectively.

3.3 Equipments

ABx pentra 60 C+ automated hemocounter from Horiba ABx diagnostics, manufactured by Vital Scientific, Ekkerstijt, Netherlands was used for the determination of hemoglobin.

Electric centrifuge 80-2 from Gen Equipment Corporation Ltd, Shanghai, China was used for sample centrifugations.

Multiscan EX Microplate Photometer, type 355 from Thermo Electron Corporation, manufactured in Shanghai, China was used for determination of hCG and Mg^{2+} was determined by selectral junior manufactured by Vital Scientific, Ekkerstijt, Netherlands.

Wellwash 4 Mk 2 Microplate Strip Washer from Thermo Electron Corporation, manufactured in Shanghai, China was used for washing wells during the determinations.

3.4 Demographics

The demographics that were recorded for each subject were maternal age, marital status, religion, ethnicity, educational background, occupation, gestational age (using the day of LMP and AUS), weight, height, blood pressure, smoking habits, intake of alcohol and supplements usage, blood group and Rhesus factor and sickling status.

3.5 Laboratory analysis

3.5.1 Hb and urine protein determination

Measurement of Hb was immediately performed after blood collection using ABx pentra 60 C+ automated hemocounter (Horiba ABx diagnostics, Ekkerstijt, Netherlands). Urine protein was also determined by the use of dip sticks (AcuBiotech Co.Ltd, Beijing Airport Industrial Zone Benijing, China).

3.5.2. hCG determination

Serum hCG was assayed using the quantitative sandwich ELISA technique using a commercial test kit obtained from GenWay Biotech, Inc., San Diego, USA on the Multiscan EX Microplate Photometer from Thermo Electron Corporation, Shanghai, China. The manufacturer's instructions were followed for the analysis. All tests were performed in duplicate with variation not exceeding 10%.

3.5.2.1 Principle of the assay:

The assay system utilizes a unique monoclonal antibody directed against a distinct determinant on the hCG. Mouse monoclonal anti- hCG antibody was used for solid phase immobilization. A goat anti whole hCG antibody was added to the antibody- enzyme (horseradish peroxidase) conjugate solution. The test sample was allowed to react sequentially with the two antibodies, resulting in the hCG molecules being sandwiched between the solid phase and enzyme- linked antibodies. After two separate 30 minutes incubations at 37^0C, the wells were washed three (3) times to remove unbound labeled antibodies. A solution development was stopped with the addition of stop solution after one hour which changed the color to yellow. The concentration of hCG was directly proportional to the color intensity of the test sample. Absorbance was measured spectrophotometrically at 450 nm.

3.5.3 Mg^{2+} determination: The serum Mg^{2+} was assayed using a commercial test kit obtained from GenWay Biotech, Inc., San Diego, USA by the use of selectra junior manufactured by Vital Scientific, Ekkerstijt, Netherlands.

3.5.3.1 Principle of the assay: Mg^{2+} ions in an alkaline medium form a coloured complex with xylidyl blue. The absorbance increase is proportional to the magnesium concentration in the sample. Glycoletherdiamine-N,N,N', N'-Tetraacetic acid (GEDTA) is used as masking agent for calcium ions. Calibrations, blanking and controls were done based on the instructions of the manufacturer. All the instructions of the manufacturer were followed.

3.6 Statistical analyses

Microsoft Excel 2007 software was used for data storage and analyses. Data on maternal age, weight and height, BMI, Hb, Urine Protein, systolic and diastolic blood pressure were presented as mean with standard deviation, medians and ranges (min and max). Concentration of Mg^{2+} and hCG were presented in mmol/ml and MoM respectively as mean with standard deviation, median and range (max and min).The results of biochemical markers were also expressed as the gestation- specific multiples of the median (MoM). MoM values were calculated by dividing the observed marker concentration by the median value for the gestational week at which the sample was obtained.

Pearson's correlation coefficient (r) was used for estimating correlations between age and concentrations of Mg^{2+} and hCG.

Student's t-test was used to test for differences between means of concentrations of Mg^{2+} and hCG at recruitment and between mean concentrations of Mg^{2+} and hCG in normal and adverse pregnancies. Means of Hb, SBP and DBP, UP, BMI, and age in the cases group were all compared with that of the controls. Statistical significance was determined at $P< 0.05$. The www.graphpad/quickcals/ttest1 online software was used in all calculations to establish the significance.

CHAPTER FOUR

4.0 RESULTS

4.1 Clinical and socio-demographic parameters of the study population

The study population was made up of 150 pregnant women in their second trimester showing features of preeclampsia (women with features of preeclampsia, WFP) and 50 controls (second trimester pregnant women with no features of preeclampsia, WNFP). The pregnant women with features of preeclampsia (WFP) were further divided into four groups depending on the level of protein present in the urine (proteinuria). A total of 87 pregnant women showed the features of preeclampsia but no detectable urine protein. Those with urine protein, assigned + 1 and + 2 were 72 and 28 respectively. Out of the total women with features of preeclampsia, 13 were diagnosed as preeclamptic patients with urine protein assigned + 3.

The clinical and the socio-demographic parameters of the entire study population are shown in Table 2. The mean age of the women with features of preeclampsia (WFP) (29.65 ± 5.24) compared with women with no features of preeclampsia, controls (27.76 ± 5.22) was statistically significance ($p < 0.05$). The body mass index (BMI), both systolic and diastolic blood pressures of WFP compared with the control were significantly elevated ($p < 0.05$) (Table 2). Among the study population, Illiteracy was higher in the subjects with preeclamptic features than non-preeclamptic control ($Z = 2.77$, $p < 0.05$). Differences in educational background of the study population were significant at the extremes ($p < 0.05$) (Table 2). Apart from the house wives who were many in the pregnant women with features of preeclampsia ($p< 0.05$), there was no statistical significant difference between the study groups occupational categories ($p > 0.05$) (Table 2).

Table 2 Clinical and Socio-demographic parameters of study population

Parameter	WFP (N=150)	Control (N=50)	95% CI of mean difference	p-value
Age (years)	29.65 ± 5.24	27.76 ± 5.22	0.20 - 3.57	0.0494*
BMI (Kg/m^2)	23.37 ± 3.5	20.97 ± 1.65	1.38 – 3.41	0.0001**
SBP (mmHg)	137.78 ± 23.73	117.80 ± 8.40	13.22 – 26.74	0.0001**
DBP (mmHg)	93.78 ± 18.46	84.48 ± 5.37	3.77 – 14.22	0.0008**
Education (%)			Z-Score	
Illitrates	59 (39.3)	10 (20.0)	2.77	0.0057**
Primary	13 (8.7)	7 (14.0)	0.97	0.3334
Secondary	42 (28.0)	18 (36.0)	1.02	0.3039
Vocational	34 (22.7)	7 (14.0)	1.44	0.1490
Tertiary	2 (1.3)	8 (16.0)		
Occupation (%)				
Trader	78 (52.0)	33 (66.0)	1.77	0.0767
Public Servant	1 (0.7)	3 (6.0)	1.53	0.1257
House wife	71 (47.3)	12 (24.0)	3.17	0.0015**
Unemployed	-	2 (4.0)	-	-

WFP = women with features of preeclampsia. Control = Women with no features of preeclampsia (WNFP). * P < 0.05, ** p < 0.01. BMI = Body Mass Index, SBP = Systolic blood pressure, DBP=Diastolic blood pressure

4.2 Comparison of serum markers and haemoglobin between WFP and controls of the study population

For the serum makers, Mg^{2+} was significantly lowered whiles hCG was significantly raised in the women with features of preeclampsia (WFP) compared with the controls counterparts ($p < 0.001$) (Table3). The mean difference of haemoglobin (Hb) concentration of the women with features of preeclampsia (WFP) compared to the controls was statistically significant ($p < 0.001$).

Table 3 Comparison of serum markers of the study population

Parameter	WFP (N=150)	Control (N=50)	95% CI of mean difference	p-value
Mg^{2+} (mmol/ml)	0.68 ± 0.11	0.87 ± 0.07	-0.22 – (-0.16)	0.0001†
hCG (MoM)	0.99 ± 0.20	0.85 ± 0.21	0.08 - 0.21	0.0001†
HB (g/dl)	10.82 ± 1.23	13.83 ± 0.81	-3.38 – (-2.64)	0.0001†

WFP = women with features of preeclampsia. Control = Women with no features of preeclampsia (WNFP). Mg2+ = Magnesium ions, hCG =Human chorionic gonadotropin, Hb=haemoglobin. †p < 0.001.

4.3 Comparison of clinical and serum markers of women with different degrees of proteinuria

The clinical, serum markers and haemoglobin levels of the pregnant women with different degrees of proteinuria were compared (Table 4). Out of the 150 pregnant women with features of preeclampsia, 37 showed no urine protein whiles 13 were diagnosed of preeclampsia by the clinician. The preeclamptic women had higher urine protein (+3) as compared to 72 and 28 women with +1 and +2 protein levels respectively. The mean age and BMI differences between the 4 groups were not statistically significant ($p < 0.05$). Both the systolic and diastolic blood pressures were significantly raised as urine protein increased ($p < 0.001$) (Table 4). Pregnant women with no urine protein (0) showed considerably normal systolic and diastolic blood pressures (118.85±10.61) and (85.36 ± 8.64) as compared to individuals with traces of urine protein (+1), (132.64 ± 24.42) and (91.44 ± 17.01) respectively ($p < 0.01$, not in Table). Women with high urine protein (+3) showed elevated systolic and diastolic blood pressures (175.92±18.01) and (122.69±15.89) than those with moderate urine protein (+2) (139.64 ± 24.42) and (95.93 ± 18.23) respectively ($p < 0.001$, not in table).

Mg^{2+} levels in the pregnant women significantly decreased with increased urine protein ($p < 0.0001$) (Table 4). Women with no urine protein showed significantly higher serum Mg^{2+} levels

(0.81 ± 0.09) than women diagnosed with preeclampsia (0.48±0.13) ($p < 0.001$, not shown). The gradual rise in level of hCG with respect to the amount of urine protein in the study population was not statistically significant ($p > 0.05$). Haemoglobin (Hb) levels decreased with an increased urine protein. Women diagnosed of preeclampsia (+3) showed significantly lower haemoglobin concentration than any of the groups with the features of preeclampsia ($p < 0.001$) (Table 4).

Table 4 Comparison of clinical and serum markers of women with different degrees of proteinuria

Parameter	Pregnant women with different degree of proteinuria (N = 150)			Preecalamptic Patient
	(0) (N = 37)	(+ 1) (N = 72)	(+2) (N = 28)	(> +3) (N =13)
Age/years	28.68 ± 5.47	29.89 ± 5.23	28.18 ± 4.66	31.77±4.53
BMI (Kg/m^2)	21.23 ± 1.92	23.18 ± 3.17	23.83 ± 3.44	28.58±3.21
SBP (mmHg)	118.85 ± 10.61	132.92 ± 19.53	139.64 ± 24.42	175.92±18.01**
DBP (mmHg)	85.36 ± 8.64	91.44 ± 17.01	95.93 ± 18.23	122.69±15.89**
Mg^{2+}(mmol/ml)	0.81 ± 0.09	0.71 ± 0.07	0.66 ± 0.10	0.48±0.13**
hCG (MoM)	0.90 ± 0.20	0.95 ± 0.15	1.02 ± 1.9	1.29±0.33
HB (g/dl)	12.82 ± 1.45	10.89 ± 1.20	10.43 ± 1.05	9.45±1.19**

BMI = Body Mass Index, SBP = Systolic blood pressure, DBP=Diastolic blood pressure. Mg2+ = Magnesium ions, hCG =Human chorionic gonadotropin, HB=haemoglobin. Urine protein level is designated 0, +1, +2 and +3 in order of amount of protein** $P < 0.001$

4.4 Parameters with p-values compared with various degrees of urine protein.

The parameters of the women with features of preeclampsia group were compared with the various degrees of urine protein. All the parameters with the exception of age showed significance with most of the various degrees of urine protein (Table 5). 0 and +3 and +1 and +3 showed statistical significance for all the parameters except age ($p < 0.05$).

Table 5 parameters with p-values compared with various degrees of urine protein.

Parameters	0 and +1	0 and +2	0 and +3	+1 and +2	+1 and +3	+2and +3
AGE	0.2626	0.6990	0.0742	0.1339	0.2278	0.0260
HB	0.0001	0.0001	0.0001	0.0782	0.0001	0.0111
SBP	0.0001	0.0001	0.0001	0.1538	0.0001	0.0001
DBP	0.0438	0.0029	0.0001	0.2482	0.0001	0.0001
BMI	0.0009	0.0031	0.0001	0.3709	0.0001	0.0002
hCG	0.1453	0.7035	0.0001	0.7552	0.0001	0.6160
Mg^{2+}	0.0001	0.0001	0.0001	0.0057	0.0001	0.0001

Various degrees of urine protein with p-values

4.5 Clinical and serum markers of diagnosed preeclamptic pregnant women compared with controls

The patients with preeclampsia were significantly older than the control ($p < 0.05$) (Table 6). All the clinical parameters, BMI, SBP and DBP were significantly elevated in the patients as compared to the controls ($p < 0.001$). In addition, Hb level was significantly lower in women showing features of preeclampsia ($p < 0.001$).

Table 6 Clinical and serum markers of diagnosed preeclamptic pregnant women compared with controls

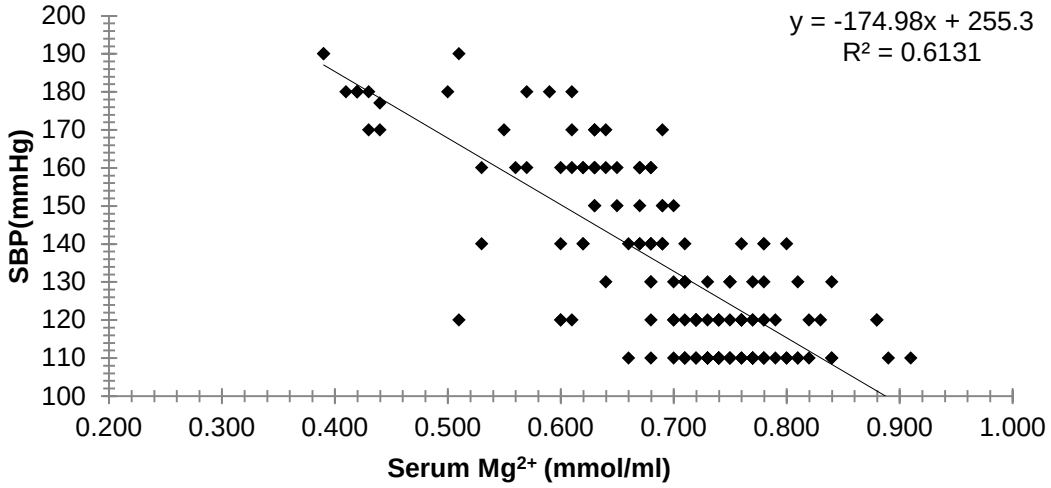
Parameter	Preeclamptic Patients (N=13)	Control (N=50)	95% CI of mean Difference	p-value
Age (years)	32.75 ± 3.28	27.96 ± 5.22	1.63-7.95	0.0036
BMI (Kg/m^2)	29.53 ± 1.64	20.97 ± 1.65	7.56-9.62	0.0001†
SBP (mmHg)	180 ± 6.77	117.80 ± 8.40	57.56-68.01	0.0001†
DBP (mmHg)	126.25 ± 9.80	84.48 ± 5.73	37.48-46.06	0.0001†
HB (g/dl)	9.36 ± 1.21	13.83 ± 0.81	-5.05-(-3.89)	0.0001†
Urine Protein	3.08 ± 0.51	-	-	-

BMI = Body Mass Index, SBP = Systolic blood pressure, DBP=Diastolic blood pressure. Mg2+ = Magnesium, HB=haemoglobin. †p < 0.001.

4.6 Association between Mg^{2+} and systolic blood pressure in pregnant women with features of preeclampsia

Figure 3 shows the relation between Mg^{2+} levels and systolic blood pressure in pregnant women with features of preeclampsia. There was a strong positive association between Mg^{2+} levels and systolic blood pressure (SBP) in pregnant women with features of preeclampsia (R^2 = 0.613, p < 0.001).

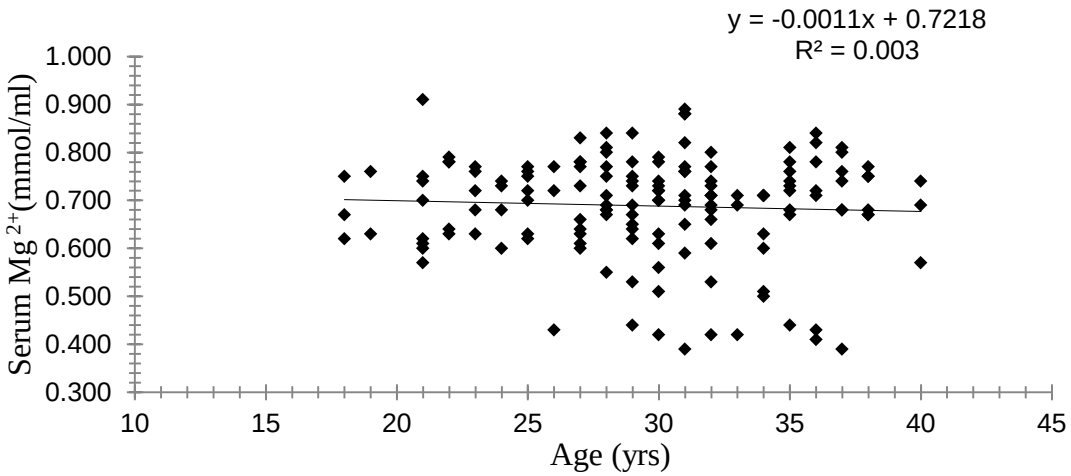
Figure3. Association between Mg^{2+} levels and systolic blood pressure



4.7 Association between serum Mg^{2+} levels and age of pregnant women with features of preeclampsia

The association between the serum Mg^{2+} levels and age of pregnant women with features of preeclampsia is shown in figure 4. There was no relationship between serum Mg^{2+} levels and age of the WFP (R^2 = 003, p > 0.05).

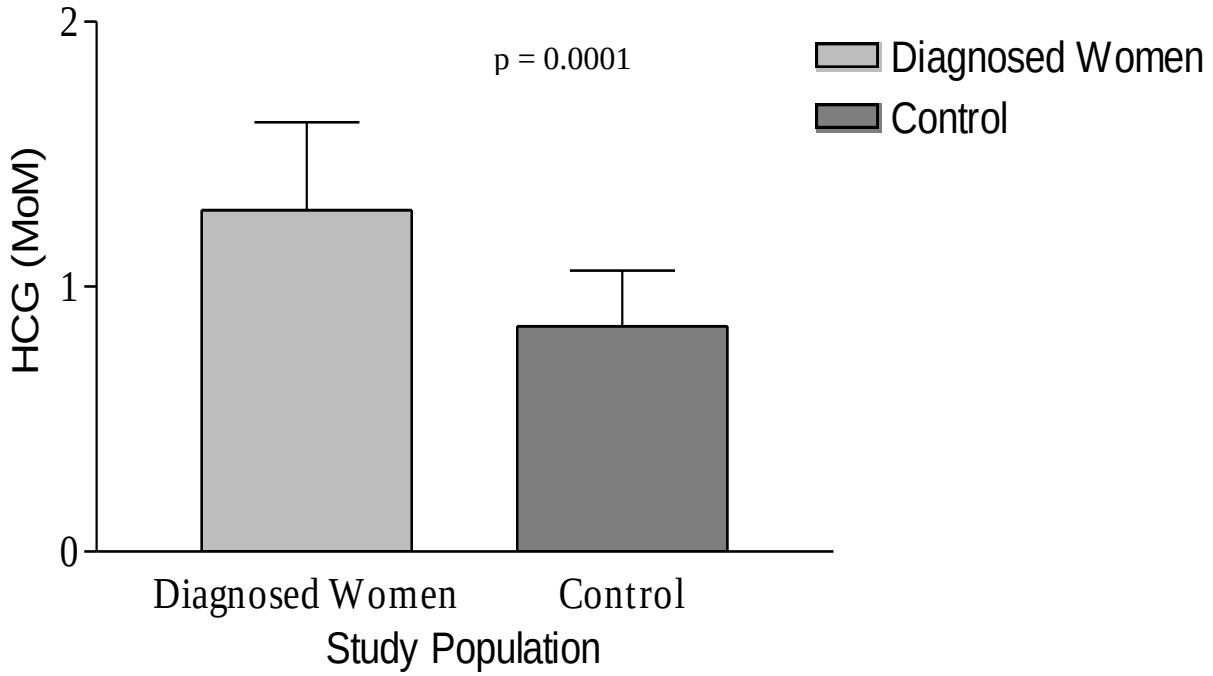
Figure 4. Association between serum Mg^{2+} level and age of pregnant women with features of preeclampsia



4.8 Comparison of serum hCG between pregnant women diagnosed of preeclampsia and control

The mean levels of hCG in the diagnosed preeclamptic pregnant women and control group are shown in figure 5. Mean hCG level in the pregnant women diagnosed of preeclampsia was significantly raised as compared with the control ($p < 0.001$).

Figure 5. Comparison of hCG levels in Diagnosed women with Control



The diagnosed preeclamptic women had relatively high levels of hCG as compared to the control. Even though none of the diagnosed preeclamptic women had hCG level above 2.0MoM, these levels were statistically significant as compared to the control ($p< 0.001$).

4.9 Association of urine protein (Proteinuria) with serum Mg^{2+}

The association of urine protein with serum Mg^{2+} level is shown in Table 7. The level of urine protein was strongly associated with serum Mg^{2+} level. Pregnant women with no traces of urine protein assigned "0" (none) were not at risk of showing low serum Mg^{2+} as compared to women with +1 (mild) state of proteinuria in the same trimester (OR = 0.05, 95% CI =0.01 – 0.21, $p < 0.0001$) (Table7). Pregnant women showing urine protein designated +2 (moderate) showed minimal risk level but not statistically significant ($p > 0.7418$) which may be of preeclampsia in the second trimester with urine protein at +2, were found to show very low serum Mg^{2+} (OR = 12.00, 95% CI =1.50-95.80, p = 0.0057).

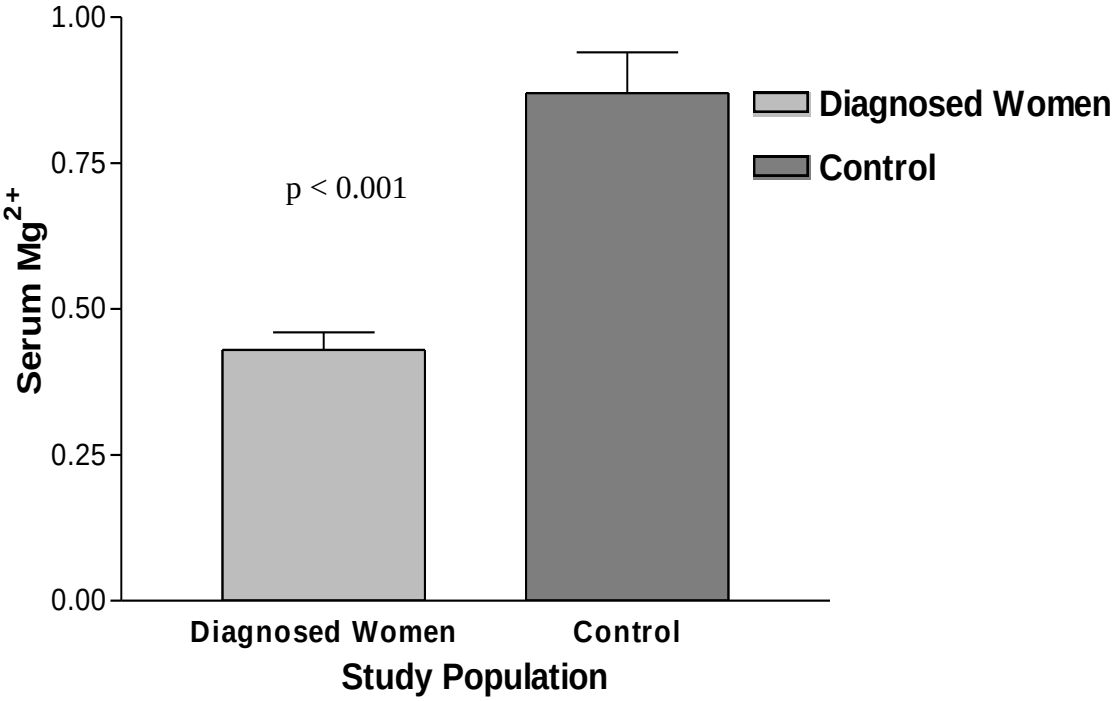
Table 7 Odds of association of urine protein (proteinuria) with serum Mg^{2+}

Proteinuria	OR	95% CI	p-value
None (0)	0.05	0.01 - 0.21	< 0.0001†
Traces (+)	1.15	0.77 - 1.50	0.7418
Moderate (+2)	2.97	1.26 - 6.99	0.0119*
High (> +3)	19.62	2.48 - 155.29	0.0001†

Urine protein level is designated 0, +1, +2 and +3 in order of amount of protein. OR = odds ratio, CI= confidence level. †$p < 0.001$ and * $p < 0.05$.

4.10 Comparison of serum Mg^{2+} level between pregnant women diagnosed of preeclampsia and control.

Figure 6 Comparison of Mg^{2+} levels in study groups



The mean levels of Mg^{2+} in the diagnosed preeclamptic pregnant women and control group are shown in Figure 6. Mean Mg^{2+} level in the pregnant women diagnosed of preeclampsia was significantly lower compared with the control ($p < 0.001$).

CHAPTER FIVE

5.0 DISCUSSIONS AND CONCLUSION

5.1 Discussions

The present study is the first research work on using multiple biochemical markers in predicting preeclampsia in Ghana. The study showed that the mean ages of the control group or Women with No Features of Preeclampsia (WNFP), Women with Features of Preeclampsia (WFP) and women who were eventually diagnosed with preeclampsia were between 27.96 and 32.75(Table 2). There was no relationship between serum Mg^{2+} levels and age of the study groups ($p > 0.05$) (fig 4). There was a strong significance however between the ages of the control and the preeclamptic women (p-value < 0.05) (Table 6). There was no statistical significance between age and all the comparisons of the various degrees of urine protein (p-value < 0.05) (Table 5). Other studies reported that maternal age between 30-40 years is among the clinical risk factors for the development of preeclampsia (Neal et al., 2004; Ecsen *et al*., 2003; N H L B I, USA 1998).

The increased incidence of preeclampsia noted among patients older than 30 years probably reflects undiagnosed chronic hypertension with superimposed PIH (Neal *et al*., 2004).

The mean BMI of this study was between 20.97 and 29.53 for all the study groups (Tables 2, 4 and 6). At all this levels it shows a very positive statistical significance (p-value< 0.001) (Tables 2, 5 and 6). Significant difference was observed at all the comparism of the various levels of urine protein with BMI (p-value < 0.001) (Table 5), except the +1 and +2 comparison. Significant difference was also observed between the BMI of the controls and the preeclamptic group (p-value < 0.001) (Table 6).

A study in the U S A showed that,preeclampsia risk is doubled from a BMI of 26 and nearly tripled at a BMI of 30 (Neal *et al.,* 2004). Similar observations were made in other studies (O'Brein, 2003; Neal et al., 2004; Ecsen *et al.,* 2003; N H L B I, USA 1998; Douglas and Redman, 1994).

BMI and preeclampsia share certain pathophysiological features, including endothelial dysfunction, oxidative stress, and an increased state of inflammation (O'Brein, 2003).

A significant difference was observed for systolic blood pressure (SBP) and diastolic blood pressure (DBP) among the study groups (p-value < 0.001) (Table 2 and 6). Results from this study showed a significant association among all the parameters and blood pressure p-value <0.001) (Tables 2 and 6). Apart from the +1 and +2 comparison, both SBP and DBP show statistical significance among all the other comparism (p-value < 0.001) (Table 5). There was a strong positive association between Mg^{2+} levels and systolic blood pressure (SBP) in pregnant women with features of preeclampsia (p < 0.001) (Fig 3).

All the 13 first time pregnant women who eventually developed preeclampsia had blood pressure greater than 160/110 mmHg, and a p-value < 0.001) (Table 6).

Several studies have highlighted the fact that in a pregnant woman whose blood pressure is greater than 160/110mmHg at the second trimester is a predictor of preeclampsia (Ustun, 2007, N H L B I, USA 1998, Neal *et al*., 2004).

During gestation, placental tissue cells called trophoblasts act like invaders, attacking the maternal blood vessels that supply blood to the embryo in an effort to draw even more nutrients to the placenta to increase the supply of nutrients.

The high blood pressure in pregnancy strains the mother's kidneys. The process of opening up the arteries fails, the fetus does not draw sufficient blood from the mother, and she may develop pre-eclampsia. This rise in blood pressure increases blood flow to the placenta, but it is dangerous for the mother (Poon *et al.*, 2010).

The formation of a uteroplacental vasculature insufficient to supply adequate blood to the developing fetus results in fetoplacental hypoxia, leading to imbalances in the release and metabolism of prostaglandins, endothelin, and nitric oxide by placental and extraplacental tissues. These as well as enhanced lipid peroxidation and other undefined factors contribute to the hypertension, platelet activation and systemic endothelial dysfunction characteristics of preeclampsia (Sibai *et al.*, 2003)

A significant difference was observed between urine protein and all the other clinical parameters (p-value < 0.001) (Table 2). There was also a significant difference between all the comparisons of urine protein and all the other parameters except age (p-value < 0.005) (Table5). With the exception of the +1 and +2 comparison all the other comparisms were statistically significant (p-value < 0.005).

The association of urine protein with serum Mg^{2+} at the +3 level showed statistical significance (p-value < 0.001) (Table 7).

All the 13 (8.7%) of the first time pregnant women who eventually developed preeclampsia had more than plus two (+2) amount of protein in their urine samples with p < 0.001(Table 6). The presence of proteinuria with excretion of 0.3 g or more of protein in a 24-hour urine specimen or

+1 or greater on two random urine samples collected four or more hours apart is an indication of preeclampsia (Neal *et al.*, 2004; Samadi, 1996, Ali *et al*., 2011).

Proteins are normally confined to the blood by the filtering role of the kidney, spilling into urine is as a result of preeclampsia which temporarily damages this "filter."The damaged filter therefore allows protein in to the urine of such pregnant women (Ferrazzani *et al*., 1994).

Even though Sibai,1996, reported that smoking and alcoholism by pregnant women increase the risk of preeclampsia, none of the pregnant women in this study admitted to either smoking or alcohol consumption perhaps because of cultural orientation. Information on those parameters was therefore not available for comparison.

Illiteracy was statistically significant between the subjects with preeclamptic features and the non-preeclamptic control ($p < 0.05$) (Table 2). Differences in educational background of the study population were significant at the extremes ($p < 0.05$) (Table 2), whiles there was statistical significant difference between the house wives in the pregnant women with features of preeclampsia and the control ($p < 0.05$), there was no statistical significant difference among the other occupational categories ($p > 0.05$) (Table 2).

Other studies also observed similar findings (Ali *et al*., 2011; Klonov *et al.*, 1996; Poon *et al.*, 2010; N H L B I, USA 1998, Fujiwara *et al*., 2004).

There is an association between educational level and antenatal care, and an influence of both education and antenatal care on maternal mortality (Ali *et al*., 2011). The illiterates been significant between the WFP and WNFP could be due to the fact that they do not attend pre-natal care as compared to the educated. The significance between the house wives and the other occupational categories could also be due to the fact that the house wives might not have been

actively involved in any work and this could lead to obesity and many other adverse pregnancy outcomes.

There was a statistical significance in comparing Hb with the other serum markers (p-value < 0.001) (Table 3).

Apart from the +1 and +2 and the +2 and +3 urine protein comparism, all the other comparisms with the mean Hb were very significant (p-value < 0.001) (Table 5).

A significant difference was observed between the mean Hb of the control group and the 13 pregnant women who developed preeclampsia (p-value < 0.001) (Table 6).

Several studies have highlighted the fact that severe anaemia (Hb < 10) is associated with a higher risk for preeclampsia and poor perinatal outcomes (Ali *et al*., 2011; Neal *et al*., 2004; Poon *et al.*, 2010; N H L B I, USA 1998).

The decreased Hb may be as a result of poor nutrition and prenatal care and non supplementation of ion during pregnancy and this can influence the hemoglobin values of especially the preeclamptic women.

There was statistical significance in the mean of hCG between the women with features of preeclampsia and the control (p-value < 0.001) (Table 3). With respect to the protein in urine comparisons, the mean of hCG was only significant at the 0 and +3 and the +1 and +3 comparisons (p-value < 0.001) (Table 5).

Mean hCG level in the pregnant women diagnosed of preeclampsia was statistically significantly as compared with the controls (p < 0.001) (Fig 5).

Several studies have highlighted the fact that serum hCG levels were significantly elevated between preeclamptic pregnant women and non preeclamptic women (Myatt and Miodov 1999, Mizelewski, 2007, Reis *et al*., 2004; and Morris *et al.*, 2008).

An elevation in serum hCG levels in the second and third trimesters has been linked to the development of preeclampsia and other adverse pregnancy outcomes. It can predict an increased risk of perinatal death, low birth weight, small-for-gestational-age infants, preterm premature rapture of membranes, and preterm birth (Myatt and Miodov,1999).

There was statistical significance in the mean of Mg^{2+} between the women with features of preeclampsia and the control (p-value < 0.001) (Table 3). With respect to the protein in urine comparisons, the mean of Mg^{2+} was the only parameter that was statistically significant at all the various degrees of urine protein comparisons (p-value < 0.001) (Table 5).

Mg^{2+} concentration was also very significant between the 13 women who were diagnosed with preeclampsia and the control (p- < 0.001) (Table 6) (Fig 6).

There was a strong positive association between Mg^{2+} levels and systolic blood pressure (SBP) in the pregnant women with features of preeclampsia group (p < 0.001) (Fig 3).

Several studies have highlighted the fact that serum Mg^{2+} levels were significantly lower between preeclamptic pregnant women and non preeclamptic women (Esen *et al*., 2003; Dahle *et al.*, 2003; Thompson *et al*., 2004; Magee *et al*., 2005; Altura *et al.*, 1994, Flowers *et al*., 1965;.Handwerker *et al*., 1996).

A deficiency in magnesium could possibly lead to spasms in the placenta and the umbilical cord. These spasms could lead to premature labor and increases the risks of birth defects or even infant mortality. Magnesium helps to relax the muscles, and getting the proper amount of magnesium

has been shown to relax the uterus thereby allowing the pregnancy to continue on as it should for the proper length of time (Dahle *et al.*, 2003).

The study shows that both markers when used together provide a better prediction of preeclampsia. This is because all the 13 pregnant women who were diagnosed with preeclampsia all had lower levels of Mg^{2+} and relatively higher level of hCG (fig 5 and 6). Individually Mg is a better predictor of preeclampsia as compared to hCG (fig 5).

5.2 Study limitations

The recruitment for the study went slowly. Most pregnant Ghanaian women are not interested in any research study. Some participant required some form of incentives before their blood samples were taken. In the present study the study design required first time pregnant women aged 16 to 40 years and free of any visible disorders and noticeable diseases, so it was difficult getting the required pregnant women to enroll. The lack of information about the risk and dangers of preeclampsia among Ghanaian pregnant women also made it very difficult in getting women to be enrolled.

The acquisition of reagents for the study was very difficult as the reagents were not readily available in Ghana. Cost of reagents was quite exorbitant as it was bought in foreign currency and we had to pay tax and duty before the reagents got to Ghana. Resources in general to enhance the smooth performance of the research was not available, as a result of this a second blood samples which was intended to be taken was not possible.

5.3 Conclusion

The study showed that Mg^{2+} and hCG can be employed as double biochemical markers for predicting pre-eclampsia with some efficiency and effectiveness as used elsewhere internationally. The study also assessed the usefulness of the markers for adverse pregnancy outcomes. The data compared favorably with other reports as preeclamptic cases were seen in the population studied. There is an indication that hCG and Mg^{2+} are both useful in preeclampsia screening. It is also seen that Mg^{2+} is a more positive predictor of preeclampsia as compared to hCG according to this study.

The study showed comparable low levels of Mg^{2+} (0.65 mmol/ml) which was not within the acceptable international levels of 0.7 to 1.0 mmol/ml. It also showed that mean concentrations of hCG (0.99MoM) of pregnant Ghanaian women are relatively low and within the international acceptable level of not more than 2.0MoM.

The study has shown that Mg^{2+} as a biochemical marker for predicting preeclampsia is a better predictor as compare to other available biomarkers in this study.

5.4 Recommendation

The study strongly proposed that Mg^{2+} be included in the ANC cases of all pregnant Ghanaian women as part of their antenatal care, especially in the second and third trimester of their pregnancy.

The daily requirement of Mg^{2+} for a pregnant woman is between 350 and 360mg. Mg^{2+} can be obtained from eating green vegetables, legumes such as beans and peas/ nuts and seeds, whole grains and many more. Since these foodstuffs are common in Ghana, pregnant women who are

found out to have lower levels of Mg^{2+} can be put on diet or encourage to eat more of these vegetables during pregnancy.

With the target of the Millennium Development Goals in sight, preeclampsia/eclampsia needs to be identified as a priority area in reducing maternal mortality in developing countries. Since the mainstay of control remains health care based strategies, national governments and supporting agencies should channel efforts at strengthening the public health systems and improving access to trained health care providers. Further research is needed to understand the causes and the best preventive strategies for preeclampsia specific to geographic areas (WHO, 2005).

Unfortunately there is no single test to predict or diagnose preeclampsia. As stated above by the WHO the determination of Mg^{2+} levels in pregnant Ghanaian women blood serum should be included in the Antenatal care (ANC) cases since the test is simply done and increasing Mg^{2+} levels is also achievable. This will help reduce the incidence of preeclampsia in Ghana. It is therefore hope that the Ghana Health Service as well as the National Health Insurance Scheme will include the determination of Mg^{2+} levels in ANC cases of all pregnant Ghanaian women especially in the second and third trimester as WHO focused antenatal care strategy in 2005 recommends screening for preeclampsia during the third antenatal visit at above 20 weeks.

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APPENDICES

A CONSENT FORM

B QUESTIONNAIRE

Appendix A

CONSENT FORM FOR THE COLLECTION OF BLOOD SAMPLE

FOR MEDICAL RESEARCH PURPOSES

TITLE OF STUDY:

MATERNAL SERUM **HUMAN CHORIONIC GONADOTROPIN (hCG) AND MAGNESIUM (Mg^{2+}) AS BIOCHEMICAL MARKERS IN PREDICTING PREECLAMPSIA IN PREGNANT GHANAIAN WOMEN.**

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DR BEN D.R.T.ANNAN OBGYN DEPT KORLE-BU. MAY, 2012

EXPLANATION TO THE MOTHER:

Our aim is to determine some identifiers for pregnant women who are at the risk of developing preeclampsia. Preeclampsia is a medical condition in which hypertension arises in pregnancy associated with high blood pressure and significant amount of protein in the urine of pregnant women. Preeclampsia is a multisystem disease of pregnancy of unknown cause. It is a maternal

syndrome, which is characterized by increased blood pressure, edema, proteinuria and abnormal clotting, liver and renal functions. We want to determine the levels of: Magnesium and Human Chorionic Gonadotropin (hCG) as markers for predicting preeclampsia among pregnant Ghanaian women, so that if they turn out to be useful we can use them for screening.

PROCEDURE:

We want to take blood sample at the time of enrollment between 16-24 weeks of gestation. We will take about 5 ml of venous blood, which equals the volume of fluid in a table spoon. This procedure is not dangerous to your health and the health of your baby.

DISCOMFORT:

Our staff is highly experienced in taking blood, so it must be safe and minimally painful to you. After we take the blood you may feel a slight burning sensation which is for a very short period and will not require any special attention or treatment.

RISKS/ DANGERS:

There are no potential risks involved to you or to your baby in this study.

RIGHT TO REFUSE OR WITHDRAW:

We will be grateful if you will agree for your blood to be studied. However, you are not obligated to. If you refuse, this will not in any way influence the way you will be treated.

BENEFITS:

At this moment, you will not have any direct benefits from participating in this study. But you may benefit in the future. For instance, if this maternal screening program is good and what we find can be used for helping to identify the high risk pregnancies and to facilitate the delivery (induction, cesarean section). Other first pregnancies may be screened by using this procedure to prevent preeclampsia.

CONFIDENTIALLITY:

All the information that you give to us and the test result will be fully confidential. No one will have access to your data except the principal investigator.

PROBLEMS OR QUESTIONS:

For any question and further information you can contact the principal investigator Mr. Mohammed Mustapha Seini and supervisors Dr.Bartholomew Dzudzor the Department of Medical Biochemistry, University of Ghana Medical School, Korle-Bu, Accra. Contact number: **0243716700** and Dr. B.R.T.Annan K B T H Accra.Contact number: **0208127176**

Consent:

I……..of …….....

……...give my consent to the research procedures above, the nature, purpose and possible consequences of which have been described to me

By…….

Patient's
signature…….Date…….....

Doctor's signature……...

Appendix B

QUESTIONNAIRE

***MATERNAL SERUM* HUMAN CHORIONIC GONADOTROPIN (hCG) AND MAGNESIUM AS BIOCHEMICAL MARKERS IN PREDICTING PREECLAMPSIA IN PREGNANT GHANAIAN WOMEN.**

1. Full name of participant ……

2. Other name, if any …….

3. ID number …… 4.Age …….

4. Marital status:

[ ] Married [ ] Single [ ] Divorced [ ] Widow

5. Religion:

[ ] Christian [ ] Muslim [ ] Other, specify

6.Tribe…….

7.Educational background:

[ ] Primary [ ] Vocational [ ] Secondary [ ] University

8. Occupation……

9. Blood group:

[ ] A [ ] B [ ] AB [ ] O

10.Rhesus factor:

[ ] Positive [ ] Negative

11.Sickling status:

[ ] Positive [ ] Negative

12. Age of first menstrual period ……

13. The day of last menstrual period ……..

14. Week of gestation (USI) ……

15. Weight (kg)……Height (cm) ……BMI …….. BP ……

16. Contact address/ phone ……

25. Do you smoke?

[ ] Yes [ ] No

26. Which best describe you?

a) Never smoked

b) Only tried smoke once

c) Used to smoke, but gave up

d) Smoke occasionally (sometimes)

e) Smoke regularly

If you answered any of 2, how many cigarettes per day do you smoke? …….

27. Are you taking any drugs or supplements?

[ ] Yes [ ] No

28. If yes, name them? …….

29. Do you know anything about preeclampsia? [ ] Yes [ ] No

30. If yes what do you know about it……

31. Has any of your relatives or friends been diagnosed of preeclampsia?

[ ] Yes [ ] No THANK YOU.