CALCIUM METABOLIC INDICES OF PREGNANT WOMEN WITH PROTEINURIC PRE-ECLAMPSIA AND ECLAMPSIA

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DEPARTMENT OF BIOCHEMISTRY,

FACULTY OF SCIENCE,

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ZARIA, NIGERIA

APRIL, 2014.

DECLARATION

I hereby declare that this thesis entitled "CALCIUM METABOLIC INDICES OF PREGNANT WOMEN WITH PROTEINURIC PRE-ECLAMPSIA AND ECLAMPSIA" was performed by me in the Department of Biochemistry under the Supervision of Dr. K.M. Anigo, Mr. O.A Owolabi and Dr. S. Adaji. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at any Institution.

_____ _____

Muhammed Shehu Hassan Date

CERTIFICATION

This thesis titled "CALCIUM METABOLIC INDICES OF PREGNANT WOMEN WITH PROTEINURIC PRE-ECLAMPSIA AND ECLAMPSIA" by Hassan, Shehu Muhammed meets the regulations governing the award of the degree of Master of Science (MSc) of Ahmadu Bello University and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This work is dedicated to my loving parents Late Mal. Hassan Lawal and Mrs. Hassana Lawal. May Allah (S.W.T) have mercy on them as they were to me while I was young. Amen.

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All acclamations and appreciation's are for almighty Allah, who bestowed mankind with knowledge and wisdom. Allah has the knowledge of known and unknown and equipped his humble creature with mental facility to search for his creation. This is his mercy and grace that enabled me to complete this work.

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ABSTRACT

This case control study aimed at evaluating the calcium metabolic indices of pregnant women with preeclampsia and eclampsia was conducted on pregnant women attending Ahmadu Bello University Teaching Hospital at Shika, Nigeria. A total of 60 pregnant women at gestational age of over twenty weeks were registered for the study after obtaining informed consent. The study subjects were divided into four groups: Two control groups (I-Pregnant normotensive & II-Pregnant hypertensive) and two case groups (III-Preeclampsia & IV-Eclampsia). Characteristics of each participant were recorded using a pre- tested semi-structured questionnaire after which 5 ml of venous blood was drawn for biochemical and haematological analysis. Results obtained showed Group IV had highest age group percentage of 60% followed by group I,II and III with 40%, 33.3% and 36% respectively, 40% of the pregnant women were housewives irrespective of their educational status and they survive on monthly income of less than ₦5000, All the subjects have formal education. Mean arterial blood pressure was significantly different ($P < 0.05$) in all the study groups and mean amount of dietary intake of energy, water, calcium and vitamin E were low in all the groups compared to the RDA. Significant differences ($P < 0.05$) were noted in most of maternal serum electrolyte concentration in the cases when compared with the control. Changes in the serum Sodium, Chloride, Potassium were not significant ($P > 0.05$) in all the groups. Groups I and II showed no difference in levels of magnesium compared to group III and IV which is significant ($P < 0.05$) when compared with the serum parathyroid hormone levels in both case and control groups which show high level of significant difference ($P < 0.05$). There was significant difference in the levels of serum ionized, non-ionized and total calcium ($P < 0.05$) between case and control groups. No significant difference was observed in serum protein levels however there was significant difference ($P < 0.05$) in WBC count between the case $9.26 \pm 0.88 \times 10^9/l$ and control $10.64 \pm 0.80 \times 10^9/l$ groups. There was a positive relationship between calcium and some other electrolyte in all the study groups. In conclusion, the results of our study propose that deficiency in Calcium and imbalanced electrolyte concentrations may add to the promotion or development of Preeclampsia/Eclampsia seen in pregnancy.

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LIST OF ABBREVIATIONS

ABBREVIATIONS	MEANING
PTH	Parathyroid Hormone
PTHrP	Parathyroid Hormone-related protein
PCV	Packed cell volume
WBC	White blood cells
RBC	Red blood cells
PLT	Platelet
MPV	Mean platelet volume
MCV	Mean corpuscular volume
MCH	Mean corpuscular hemoglobin
MCHC concentration	Mean corpuscular hemoglobin
RDW	Red cell distribution width
LPCR	Large platelet concentration ratio
PDW	Platelet distribution width
PCT	Procalcitonin
PE	Preeclampsia
eNOS	Nitric oxide synthase
GFR	Glomerular filtration rate
PIGF	Placental growth factor
VEGF	Vascular endothelial growth factor
NK	Natural Killer
EVT	Extravillous trophoblast
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

Hypertension complicates approximately 10% of all pregnancies worldwide, and pre-eclampsia and eclampsia are major causes of maternal and perinatal morbidity and mortality (Duley, 2009). Currently, pre-eclampsia and gestational hypertension are considered either separate diseases affecting the same organs or different levels of severity of the same underlying disorder (Villar, 2006). Based on available estimates and case–fatality rates, each year up to 40 000 women, mostly in developing countries, die of hypertensive disorders (Villar, 2004). Pre-eclampsia and eclampsia complicate 2–8% of pregnancies and, overall, 10–15% of direct maternal deaths are associated with these conditions (Duley, 2009).

Calcium as a nutrient is most commonly associated with the formation and metabolism of bone. Over 99 percent of total body calcium is found as calcium hydroxyapatite ($Ca_{10}[PO_4]_6[OH]_2$) in bones and teeth, where it provides hard tissue with its strength. Calcium in the circulatory system, extracellular fluid, muscle, and other tissues is critical for mediating vascular contraction and vasodilatation, muscle function, nerve transmission, intracellular signaling, and hormonal secretion. Bone tissue serves as a reservoir for and source of calcium for these critical metabolic needs through the process of bone remodeling.Calcium absorption increases early in pregnancy. At the same time, the protein-bound fraction in serum usually declines gradually throughout pregnancy, whereas the free ionic concentration remains relatively constant (Pitkin, 1985).

Pre-eclampsia is a complex pregnancy complication associated with increased blood pressure (Davey and MacGillivary, 1988). This condition seems to be linked to

oxidative stress within the placenta. Increased production of lipid peroxides, thromboxane and/or cytokine triggered vascular and organic dysfunctions have been observed in pre-eclampsia (Lefèvre *et. al.,* 1997). It is one of the causes of high morbidity for both mother and fetus, especially in developing countries (Vanderjagt *et. al.*,2004) and is characterized by hypertension, proteinuria, and edema. Without intervention, pre-eclampsia progresses to eclampsia; characterized by malignant hypertension and convulsions, requiring emergency caesarian section (Packer, 2005).

The indicated delivery of women to prevent the progression of pre-eclampsia is responsible for 15% of all preterm births (Golderberg and Rouse, 1998). The cause of pre-eclampsia remains largely unknown, but poor placentation is an important predisposing factor. A shallowly implanted placenta which becomes hypoxic, leading to an immune reaction characterized by secretion of up-regulated inflammatory mediators from the placenta, and acting on the vascular endothelium. The shallow implantation is thought to stem from the maternal immune system's response to the placenta. Not all women with reduced placental perfusion develop pre-eclampsia. Other conditions recognized to increase the risk of pre-eclampsia include obesity (Sibai *et al*., 1995), hypertension (Caritis *et al*., 1998), diabetes (Caritis *et al*., 1998), hyperhomocysteinemia (Powers *et al*., 2001), increased androgens (Laivuori *et al*., 1998), and black race (Eskenazi *et al*., 1993). Of course, these are all risk factors for cardiovascular disease in later life. Appreciation that pre-eclampsia is a multi-systemic syndrome characterized by vasoconstriction, metabolic changes, endothelial dysfunction, activation of the coagulation cascade, and increased inflammatory response, to mention only some of the organ systems involved, has redirected research (Roberts *et al*., 2003). As a result, progress in understanding the disorder has accelerated greatly with attendant optimism for potentially effective treatments (Roberts

and Gammills, 2005). Pre-eclampsia is associated with haematological complications. Altered erythrocyte aggregation has been observed in severe pre-eclampsia than in normal pregnancy (Sengupta, 1995). Despite known risk factors, it is not possible to predict the women that will develop pre-eclampsia during pregnancy. Reproductive implications of pregnancy complicated by hypertensive disorder are well known, and women are advised regarding risk of pre-eclampsia in subsequent pregnancies (Chambers *et. al.*, 2001 and Anon, 2006).

1.1 Statement of Research Problem

Approximately 287,000 women die annually from pregnancy-related complications, 85% of these deaths are in the developing countries. Out of these maternal deaths, 13% are in women with Pre-eclampsia and Eclampsia (WHO, 2010).In Nigeria approximately 37,000 women die annually because of Pre-eclampsia/eclampsia-related complication (WHO, 2004). The aetiology and pathophysiology of pre-eclampsia/eclampsia remain incompletely understood, despite its huge impact on maternal and newborn health (Alisi *et al.,* 2011). And hence the need to study the possible alterations in some parameters related to calcium metabolism in patients with pre-eclapmsia and eclampsia.

1.2 Justification

There is still paucity of knowledge on the aetiology, pathophysiology and data on dietary intake of calcium and the connection to Pre eclampsia/ Eclampsia taxomia (PET).Nigeria incidentally bears disproportionate burden of pre-eclampsia and eclampsia and its consequences but little or no data exist on the hormonal regulation of calcium and its metabolites among preeclampsia/eclampsia patients in Nigeria

In some other stress associated diseases, dramatic alteration in white blood cell values had been observed but this has not been investigated in pre-eclampsia (Oishi *et al.*, 1999)

In order to shed some light on this, this research work is designed to find any relationships between levels of calcium metabolic indices in pre-eclampsia and eclampsia among those at risk of developing the disease.

1.3 Null Hypothesis

There is no difference in the levels of calcium and its metabolites between pregnant women with preeclampsia and eclampsia and apparently healthy pregnant women.

1.4 Aim

The aim of this work is to study calcium metabolic indices among pregnant women in order to further ascertain the current evidence based interventions on its role in the management and prevention of preeclampsia and eclampsia.

1.5 Specific Objectives

The specific objectives are as follows:

i. Document the socio-economic/demographic characteristics and Blood pressure of pregnant women with proteinuric Pre-eclampsia and Eclampsia

ii. Assess dietary intake of pregnant women with proteinuric Pre-eclampsia and Eclampsia.

iii. Determine the levels of serum electrolyte and parathyroid hormone in pregnant women with proteinuric Pre-eclampsia and Eclampsia.

iv. Determine the levels of Serum Protein and some Haematological parameters of pregnant women with proteinuric Pre-eclampsia and Eclampsia.

v. Determine the relationships between serum electrolyte and calcium levels of pregnant women with proteinuric Pre-eclampsia and Eclampsia.

CHAPTER 2

LITERATURE REVIEW

2.1 History of Preeclampsia and Eclampsia

The French physician Francois Boissier de Sauvages de Lacroix in 1739 was the first to coin the term eclampsia, which he described as an acute form of convulsion, and contrasted with the chronic condition now known as epilepsy. He is credited with establishing the first methodical nosology for disease, and described several species of the genus eclampsia, one of which was *eclampsia parturientum*. Given the dramatic nature of the condition, it is perhaps surprising that more has not being written in historic literature about eclampsia. This may be explained by the fact that delivery was historically being the domain of the midwife: doctors, who in those days were exclusively men, were excluded from the scene (Mandy, 2010).

2.2 Calcium Metabolism

The pregnant woman's body provides 25 to 30 g of calcium to support the developing fetal skeleton (Trumbo and Ellwood, 2007). Much of the fetal calcium demand occurs in the third trimester of pregnancy, when the fetal skeleton undergoes mineralization. Pregnancy related changes in maternal calcium regulation, as well as contributions from the feto-placental unit and its mediators, are essential to meeting fetal calcium requirements without depleting the maternal skeleton (Trumbo and Ellwood, 2007).

Characteristics of calcium physiology during pregnancy can be summarized as follows:

- Total calcium concentration falls because of physiologic hypoalbuminemia
- Free ionized calcium concentration does not change

- The placental produces 1,25-dihydroxyvitamin D, which results in increased intestinal absorption of calcium
- Calcium is actively transported across the placenta, facilitated by parathyroid hormone-related peptide (Hofmeyr *et al.*, 2007)

Calcium homeostasis is a function of dietary intake, intestinal absorption, skeletal accretion and resorption, and urinary excretion. The key organs involved in the changes in calcium metabolism during pregnancy include the maternal parathyroid gland, the maternal intestine, and the fetoplacental unit. An increase in 1, 25-dihydroxyvitamin D, derived primarily from the placenta, stimulates intestinal absorption of calcium and phosphate, and appears to be the key regulatory factor during pregnancy. Maintenance of fetal calcium homeostasis largely depends upon parathyroid hormone-related peptide (PTHrP), which regulates active placental calcium transfer. The oscillations in these and other regulators throughout pregnancy accommodate the calcium requirements at each stage of fetal development. (Hofmeyr *et al.*, 2010)

2.3 Calcium concentration

About 40 percent of calcium ions are circulated in the plasma bound to albumin and other proteins, 10 percent are complexed with other ions, while 50 percent circulates in the free form. It is this "free" Ca^{2+} ion that is metabolically active. The active, ionized calcium level does not change significantly during pregnancy, although total serum calcium declines across gestation (Seely *et al*., 1997). The decrease in total serum calcium concentration is likely an effect of plasma volume expansion, leading to decreased concentrations of binding proteins (Seki *et al*., 1991).

2.3.1 Calcium absorption and excretion

Maternal calcium absorption from the gut increases significantly in the second and third trimesters, and then declines to pre pregnancy levels with lactation and weaning. Increased intestinal calcium absorption satisfies the fetal demand to mineralize the skeleton, while sparing to some extent the calcium contained in the maternal skeleton. Maternal urinary calcium excretion is increased throughout pregnancy (Gertner *et al*., 1986). This combination of increased calcium absorption and excretion has resulted in the term "absorptive hypercalciuria" to describe the changes in calcium metabolism during pregnancy (Gertner *et al*., 1986).

2.3.2 Role of 1, 25-dihydroxyvitamin D

1, 25-dihydroxyvitamin D is the main stimulus for increased intestinal calcium absorption. The level of 1, 25-dihydroxyvitamin D rises dramatically during pregnancy (Gertner *et al*., 1986). In the nonpregnant individual, conversion of 25-hydroxyvitamin D to its active form 1, 25-dihydroxyvitamin D via the enzyme 1-alpha-hydroxylase is generally thought to occur primarily in the kidneys, stimulated primarily by parathyroid hormone (PTH). Levels of phosphorus, estrogen, calcitonin, calcium, calcitriol (by feedback inhibition) and other factors also play regulatory roles. In contrast to the importance of PTH in 1, 25-dihydroxyvitamin D regulation in non-pregnant individuals, the increase in 1, 25-dihydroxyvitamin D during gestation does not follow an increase in PTH (Ardawi *et al.*, 1997). Intact PTH levels decline in the first half of pregnancy, reaching a nadir in the second trimester, and then rising thereafter (Ardawi *et al.*, 1997).

2.3.3 Calcium and Vitamin D requirements

Calcium

The Institute of Medicine of the National Academy of Sciences (IOM) conducted an extensive review of the available literature and, in 2010, recommended an average daily intake of 1300 mg of calcium for girls ages 9 to 18 and 1000 mg per day for women ages 19 to 50. Safe upper limits for calcium were defined as 3000 mg daily for girls ages 9 to 18 and 2500 mg daily for women ages 19 to 50, but 2000 mg for women over age 50 and for men. Their recommendations are the same during pregnancy and lactation (Abrams, 2011).

Vitamin D

Vitamin D deficiency is common throughout the world, with prevalence related to sun exposure, dietary vitamin D intake (fish or fortified milk), skin pigmentation, and obesity (Lips, 2007). Areas with a high prevalence of vitamin D deficiency include southern Europe, the Middle East, India, China and Japan (Lips, 2007).

Vitamin D sufficiency is assessed by measurement of serum 25-hydroxyvitamin D. The optimal level of serum 25-hydroxyvitamin D is controversial, but increasing data support a level of ≥30 ng/mL (75 nmol/mL) as a lower limit of optimal (Abrams, 2011). This is the target serum 25-hydroxyvitamin D level for all healthy individuals defined by the IOM. The third National Health and Nutrition Examination Survey (NHANES III) revealed hypovitaminosis D (defined as a serum 25-hydroxyvitamin D level ≤37.5 nmol/L) in 42 percent of African American and 4 percent of white American reproductive age women (Nesby *et al.*, 2002). Using 75 nmol/mL threshold as the lower limit of normal, 69 percent of pregnant women and 78 percent of nonpregnant women aged 13-44 years were deficient (Ginde, 2010). Much of the difference between the two

groups was explained by differences in vitamin D intake (primarily from fortified cereal and milk) and sun exposure (Nesby *et al.,* 2002).

The 2010 IOM report suggested the recommended daily allowance of vitamin D is 600 international units daily for all reproductive-age women, including during pregnancy and lactation (Abrams, 2011). However, it may behoove physicians to treat with vitamin D doses of 800 to 1000 international units/day, as suggested in many articles, to achieve a level of ≥30 ng/mL (Holick *et al.,* 2011).These recommended doses are unlikely to lead to vitamin D excess (defined by the IOM as a serum 25-hydroxyvitamin D level >50 ng/mL) and appear to raise 25-hydroxyvitamin D levels between the first and third trimester (Ginde *et al.,* 2010). The intake at which the dose of vitamin D becomes toxic is not clear, but a tolerable upper limit of dosing was set by the IOM at 4000 international units per day (Abrams, 2011).

The United States Food and Drug Administration (FDA) concluded the available evidence did not support a correlation between maternal calcium intake and preeclampsia (Trumbo and Ellwood, 2007). A subsequent Cochrane Review of the available data concluded that calcium supplementation reduced the risk of preeclampsia by half, and was relatively safe (Hofmeyr *et al.,* 2010). However, this meta-analysis included many studies from nutritionally deprived women living in developing countries. This analysis and others have suggested that the reduction in preeclampsia occurs primarily in women at high risk of preeclampsia and who are calcium deficient. Women with preeclampsia have reduced urinary calcium excretion, which may reflect lower intestinal calcium absorption due to low 1,25-dihydroxyvitamin D (Halhali *et al.,* 2007). A connection between vitamin D deficiency and preeclampsia has been demonstrated (Baker *et al.,* 2010), but a causal relationship has not yet been

demonstrated. Further investigation is needed to better clarify the pathophysiology of preeclampsia and its relationship to calcium and vitamin D metabolism.

There is emerging evidence to support a connection between maternal vitamin D deficiency and an increased risk for gestational diabetes mellitus. This increased risk exists even after controlling for body mass index, age, and family history of diabetes (Bodnar *et al.,* 2010). Additional research is needed to understand this connection, including interventional studies where vitamin D deficiency is prevented through adequate supplementation. Vitamin D deficiency in early pregnancy has also been linked to an increased risk of preeclampsia (Powe *et al.,* 2010).

Research on the effect of vitamin D supplementation in pregnant women reveals that there is a strong relationship between maternal and neonatal vitamin D sufficiency at delivery. Vitamin D status, in turn, influences neonatal serum calcium and PTH levels. Neonates of vitamin D deficient mothers may have abnormally low serum calcium levels, and PTH levels above normal, a pattern consistent with secondary hyperparathyroidism (Bodner *et al.,* 2010). Infants born to African-American women have significantly lower 25-hydroxyvitamin D levels than do their Caucasian counterparts, corresponding to the higher incidence of vitamin D deficiency in the African-American mothers (Leffelaar, 2010). In a longitudinal study, women with reduced concentrations of maternal 25-hydroxyvitamin D during late pregnancy and reduced levels of umbilical-venous calcium had offspring with a reduction in bone mass at nine years of age; however, a causal association was not proven (Poel *et al.,* 2012).

Multivitamin use during pregnancy may not be sufficient to correct preexisting maternal vitamin D deficiency or to prevent neonatal vitamin D deficiency (Zhang *et al*., 2008). In addition, studies of supplementation of deficient women have shown that dosages as

high as 1600 IU per day over the course of a pregnancy are inadequate to correct vitamin D deficiency. More research is needed before specific guidelines can be made regarding repletion of vitamin D in deficient pregnant women.

Causes of hypercalcemia — the upper limit of normal for total serum calcium is 9.5 mg/dL in pregnancy. Causes of hypercalcemia in pregnant and postpartum women are listed below.

Common:

- Primary hyperparathyroidism
- Immobilization (eg, bedrest)

Rare:

- Familial hypocalciuric hypercalcemia
- Postpartum hypercalcemia in hypoparathyroidism
- PTHrP-induced hypercalcemia
- Malignancy
- Thyrotoxicosis
- Adrenal insufficiency
- Vitamin A or D overdose
- Drugs (eg, thiazide diuretics, lithium)
- Milk alkali syndrome
- Acute and chronic renal failure

- Total parenteral nutrition
- Granulomatous disease (eg, sarcoidosis, tuberculosis, histoplasmosis, coccidioidomycosis)

Causes of hypocalcemia — the lower limit of normal calcium levels in pregnancy is 8.1 mg/dL. Causes of hypocalcemia in pregnant and postpartum women are listed below.

Hypoalbuminemia

- Hypoparathyroidism
- Pseudohypoparathyroidism
- Magnesium sulfate therapy
- Disorders of magnesium metabolism
- Vitamin D deficiency
- Sepsis
- Surgery
- Chemotherapy
- Fluoride poisoning
- Pseudohypocalcemia related to gadolinium
- Blood transfusion

2.3.4 Parathyroid Hormone

Parathyroid hormone (PTH) is a polypeptide secreted from the parathyroid glands in response to a decrease in the plasma concentration of ionized Ca^{2+}. This change is sensed by a specific Ca^{2+}-sensing protein in the cell membrane of the parathyroid cells (Brown and Hebert, 1996).The receptor permits variations in the plasma Ca^{2+} concentration to be sensed by the parathyroid gland, leading to the desired changes in PTH secretion. Polymorphisms of this receptor may underlie a significant portion of the variability observed in the serum calcium concentrations in normal individuals (Cole *et al.*, 1999), while inactivating mutations lead to hypercalcemia because a higher plasma Ca^{2+} concentration is required to activate the receptor and suppress PTH release (Rose and Hebert, 2000).

PTH acts to increase the plasma Ca^{2+} concentration in three ways:

- In the presence of permissive amounts of vitamin D, it stimulates bone resorption, resulting in the release of calcium phosphate.
- It enhances intestinal Ca^{2+} and phosphate absorption by promoting the formation within the kidney of calcitriol (1,25 dihydroxycholecalciferol), the major active metabolite of vitamin D.
- It augments active renal Ca^{2+} reabsorption.

These effects are reversed by a small elevation in the plasma Ca^{2+} concentration, which lowers PTH secretion.

PTH also influences phosphate balance, although its actions may be offsetting. It tends to increase phosphate entry into the extracellular fluid by its effects on bone and intestinal absorption. However, PTH also reduces proximal tubular phosphate

reabsorption, resulting in enhanced excretion. The urinary effect usually predominates in patients with relatively normal renal function, as PTH tends to lower the plasma phosphate concentration.

Renal calcium and phosphate handling — The renal effects of PTH are in part mediated by activation of specific adenyl cyclase systems in the proximal tubule and the early cortical distal nephron, including the cortical thick ascending limb, the distal tubule, and the connecting segment (Kurokawa, 1987). Activation of phospholipases C and A_2 and the subsequent breakdown of phosphatidylinositol also mediates some of the actions of PTH, particularly the reduction in proximal phosphate reabsorption (Sheu *et al.*, 1997). Stimulation of phospholipase C in the proximal tubule occurs at a lower and more physiologic concentration of PTH than stimulation of adenyl cyclase (Murer *et al.*, 1991).

PTH diminishes the proximal reabsorption of phosphate by decreasing the activity of the type II Na^+-phosphate cotransporter in the luminal membrane (Pfister *et al.*, 1997); as a result, luminal phosphate is less able to enter the cells and be returned to the systemic circulation

In comparison, the stimulatory effect of PTH on Ca^{2+} reabsorption occurs primarily in the early cortical distal nephron, particularly the distal tubule and connecting segment (Friedman and Gesek 1993). A clinical example of the importance of the interaction between PTH and calcitriol on renal Ca^{2+} handling occurs in hypoparathyroidism, a disorder in which both PTH and calcitriol levels are reduced (Kurokawa, 1987). The ensuing decrease in distal Ca^{2+} reabsorption results in persistent Ca^{2+} excretion, despite the presence of a plasma Ca2+ concentration that may be below 7.5 mg/dL (normal equals 8.5 to 10.5 mg/dL). If calcitriol is given in this setting to raise the plasma Ca^{2+}

concentration, there will still be PTH deficiency and a persistent partial defect in distal Ca^{2+} reabsorption. As a result, it may not be possible to safely raise the plasma Ca^{2+} concentration much above 8 mg/dL without inducing marked hypercalciuria that can predispose to calcium stone formation.

Acid-base balance — PTH also may contribute to the regulation of acid-base balance in the presence of an acid load. A fall in extracellular pH stimulates PTH secretion and the ensuing increase in phosphate excretion can appropriately enhance net acid excretion by buffering secreted H^+ ions (Cassola *et al.*, 1993). PTH also minimizes the fall in pH by a second mechanism, promoting bone buffering of the excess acid.

2.4 Incidence of Pre-eclampsia

The incidence of pre-eclampsia varies greatly worldwide. WHO estimates the incidence of pre-eclampsia to be seven times higher in developing countries (2.8% of live births) than in developed countries (0.4%), which is due to poor health seeking behaviours, availability of health care facilities and personnel (Dolea and AbouZahr, 2003). In Nigeria, the incidence is higher in the Northern part of the country with prevalence rate of 17%. (FMOH, 2009), compared to the Southern part with prevalence rate of 6.3% (FMOH, 2009).

Reliable statistics about women dying due to eclampsia are difficult to obtain because of the poor quality of vital statistics registration systems and hospital records in many developing countries. In addition, a sizeable number of delivery take place at home, and there are no records at all for these births. Therefore, data on women who die from eclampsia are only available from a limited number of countries Nevertheless, it is clear that the case fatality rates for eclampsia vary greatly across countries, with the risk of

death from eclampsia being much higher in developing countries than in developed ones (Aaserud *et al*., 2005)

2.5 Progression of Pre-eclampsia to Eclampsia

Only a relatively small proportion of all women with pre-eclampsia progresses to the more potentially deadly eclampsia. However, a pre-eclamptic woman in a developing country is three times more likely to progress to eclampsia than a woman in a developed country. The WHO estimates that eclampsia develops in 2.3% of pre-eclamptic women in the developing world, compared with 0.8% of pre-eclampsia cases in developed countries (RCOG, 2013).

2.6 Pathogensis of Preeclampsia

The pathophysiology of preeclampsia likely involves both maternal and fetal/placental factors. Abnormalities in the development of placental vasculature early in pregnancy may result in relative placental under perfusion/hypoxia/ischemia, which then leads to release of antiangiogenic factors into the maternal circulation that alter maternal systemic endothelial function and cause hypertension and other manifestations of the disease. However, the molecular basis for placental dysregulation of these pathogenic factors remains unknown, and the role of angiogenic proteins in early placental vascular development are under investigation. Our current understanding of mechanisms causing the pathologic changes observed in preeclampsia includes (Meher and Duley, 2005):

2.6.1 Abnormal development of the placenta

The critical role of the placenta in the pathophysiology of preeclampsia is supported by epidemiologic and experimental data that show:

- Placental tissue is necessary for development of the disease, but the fetus is not (Moore-Maxwell and Robboy, 2004)

- Preeclampsia is always cured after delivery of the placenta

Examination of human placenta at various stages of gestation in women with normal pregnancies, as well as those with preeclampsia, has led to an understanding of normal placental morphology and pathologic changes in the uteroplacental circulation that are likely to be relevant to preeclampsia.

Abnormal remodeling of spiral arteries — In normal pregnancies, the cytotrophoblast cells of the developing placenta migrate through the decidua and part of the myometrium to invade both the endothelium and highly muscular tunica media of the maternal spiral arteries, the terminal branches of the uterine artery that supply blood to the developing fetus/placenta. As a result, these vessels undergo transformation from small muscular arterioles to large capacitance vessels of low resistance, thus greatly facilitating blood flow to the placenta compared with other areas of the uterus (Zhou, *et al*., 1997). Remodeling of the spiral arteries probably begins in the late first trimester and is completed by 18 to 20 weeks of gestation, although the exact gestational age at which trophoblast invasion of these arteries ceases is unclear.

By comparison, in preeclampsia, cytotrophoblast cells infiltrate the decidual portion of the spiral arteries, but fail to penetrate the myometrial segment (Meekins *et al.*, 1994). The spiral arteries fail to develop into large, tortuous vascular channels created by replacement of the musculoelastic wall with fibrinoid material; instead, the vessels remain narrow, resulting in placental hypoperfusion. This defect in deep placentation has been associated with development of multiple adverse pregnancy outcomes, including 2nd trimester fetal death, placental infarcts, abruptio placentae, preeclampsia

with or without intrauterine growth restriction, intrauterine growth restriction without maternal hypertension, premature rupture of membranes, and preterm labor (Brosens *et al.*, 2011).

It is not known why the normal sequence of events in development of the uteroplacental circulation does not occur in some pregnancies. Vascular, environmental, immunological, and genetic factors all appear to play a role (Ilekis *et al.*, 2007).

Defective trophoblast differentiation — Defective differentiation of trophoblast is one possible mechanism responsible for defective trophoblast invasion of the spiral arteries (Eric *et al.*, 2010). Trophoblast differentiation during endothelial invasion involves alteration in expression of a number of different classes of molecules, including cytokines, adhesion molecules, extracellular matrix molecules, metalloproteinases, and the class Ib major histocompatibility complex molecule, HLA-G (Lim *et al.*, 1997). During normal differentiation, invading trophoblasts alter their adhesion molecule expression from those that are characteristic of epithelial cells (integrin alpha6/beta1, alphav/beta5, and E-cadherin) to those of endothelial cells (integrin alpha1/beta1, alphav/beta3, and VE-cadherin), a process referred to as pseudo-vasculogenesis (Zhou, *et al*., 1997). Trophoblasts obtained from women with preeclampsia do not show upregulated adhesion molecule expression or pseudo-vasculogenesis. The resulting impaired placentation and accompanying ischemia are thought to be the primary events leading to placental release of soluble factors that cause systemic endothelial dysfunction resulting in the preeclamptic phenotype.

Hypoperfusion, hypoxia, ischemia — Hypoperfusion appears to be both a cause and a consequence of abnormal placental development. A causal relationship between poor placental perfusion, abnormal placental development, and preeclampsia is supported by

the following examples: (a) Animal models that have successfully reproduced at least some of the findings of preeclampsia have involved mechanically reducing uteroplacental blood flow (Casper and Seufert, 1995), (b) Medical conditions associated with vascular insufficiency (eg, hypertension, diabetes, systemic lupus erythematosus, renal disease, acquired and inherited thrombophilias) increase the risk of abnormal placentation and preeclampsia (ACOG, 2002), and (c) obstetrical conditions that increase placental mass without increasing placental blood flow (eg, hydatidiform mole, hydrops fetalis, diabetes mellitus, twin gestation) result in relative ischemia and are associated with preeclampsia (Dekker, 1999).

Hypoperfusion is also a result of abnormal placental development. Hypoperfusion becomes more pronounced as pregnancy progresses since the abnormal uterine vasculature is unable to accommodate the normal rise in blood flow to the fetus/placenta with increasing gestational age (Gerretsen *et al.*,1981). Late placental changes consistent with ischemia include atherosis (lipid-laden cells in the wall of the arteriole), fibrinoid necrosis, thrombosis, sclerotic narrowing of arterioles, and placental infarction (ACOG, 2002). Although all of these lesions are not uniformly found in patients with preeclampsia, there appears to be a correlation between the severity of the disease and the extent of the lesions (Salafia *et al.*, 1998).

Hypoperfusion, hypoxia, and ischemia is a critical component in the pathogenesis of preeclampsia because the hypoperfused, ischemic placenta elaborates a variety of factors into the maternal bloodstream that alter maternal endothelial cell function and lead to the characteristic systemic signs and symptoms of preeclampsia (Yinon *et al.*, 2008).

2.6.2 Immunologic Factors

The focus on immunologic factors as a possible contributor to abnormal placental development was based, in part, upon the observation that prior exposure to paternal/fetal antigens appears to protect against preeclampsia (Einarsson *et al.,* 2003). Immunologic abnormalities, similar to those observed in organ rejection graft versus host disease, have been observed in preeclamptic women (Gleicher, 2007). The extravillous trophoblast (EVT) cells express an unusual combination of HLA class I antigens: HLA-C, HLA-E, and HLA-G. Natural killer (NK) cells that express a variety of receptors (CD94, KIR, and ILT) known to recognize class I molecules infiltrate the maternal decidua in close contact with the EVT cells (Loke and King 2000). Interaction between NK cells and EVT cells has been hypothesized to control placental implantation. In preeclampsia, conflict between maternal and paternal genes is believed to induce abnormal placental implantation through increased NK cell activity.

Placental bed biopsies from women with preeclampsia have revealed increased dendritic cell infiltration in preeclamptic decidual tissue (Huang *et al.,* 2008). The dendritic cells are an important initiator of antigen-specific T-cell responses to transplantation antigens. It is possible that increased number of dendritic cells may result in alteration in presentation of maternal and fetal antigens at the decidual level, leading to either abnormal implantation or altered maternal immunologic response to fetal antigens. However, definitive evidence for this theory is lacking. Genetic studies looking at polymorphisms in the killer immunoglobulin receptors (KIR) on maternal NK cells and the fetal HLA-C haplotype suggest that women with KIR–AA genotype and fetal HLA-C2 genotype were at greatly increased risk of preeclampsia (Hiby *et al.,* 2004). A systematic review found no clear evidence that one or several specific HLA alleles were involved in the pathogenesis of preeclampsia (Saftlas *et al.,* 2005).In there reports the

authors suggested that interaction between maternal, paternal, and fetal HLA types, rather than any individual genotype alone, should be an important factor to consider when studying immunogenetic determinants of preeclampsia.

A more promising finding is that patients with preeclampsia have increased levels of agonistic antibodies to the angiotensin AT-1 receptor. This antibody can mobilize intracellular free calcium and may account for increased plasminogen activator 1 production and shallow trophoblast invasion seen in preeclampsia (Thway *et al.*, 2004). In addition, angiotensin AT-1 receptor antibody stimulates sFlt-1 secretion (Zhou *et al.*, 2008). It is unclear if these alterations are pathogenic or epiphenomena.

2.6.3 Increased sensitivity to Angiotensin II

Increased sensitivity to angiotensin II has been described in preeclampsia (Granger *et al.*, 2001), and may be related to increased bradykinin (B2) receptor upregulation in preeclamptic patients. Upregulation leads to heterodimerization of B2 receptors with angiotensin II type I receptors (AT1), and this AT1/B2 heterodimer increases responsiveness to angiotensin II in vitro. As discussed above, patients with preeclampsia have increased levels of agonistic antibodies to the angiotensin AT-1 receptor. Angiotensin II is the endogenous ligand for the AT-1 receptor, thus increased activation of this receptor by auto-antibodies could induce the hypertension and vascular injury observed in preeclampsia. Studies in mice support this theory (Zhou *et al.*, 2009).

2.6.4 Genetic Factors

Although most cases of preeclampsia are sporadic, genetic factors are thought to play a role in disease susceptibility (Lachmeijer *et al.*, 2002). A genetic predisposition to preeclampsia is suggested by the following observations:

- Primigravid women with a family history of preeclampsia (eg, affected mother or sister) have a two- to five-fold higher risk of the disease than primigravid women with no such history (Lachmeijer *et al.,* 2002). The maternal contribution to development of preeclampsia can be partially explained by imprinted genes (Carr *et al.,* 2005). In a study of sisters with preeclampsia, it was demonstrated that the mother developed preeclampsia only when the fetus/placenta inherited a maternal STOX1 missense mutation on 10q22; when the fetus/placenta carried the imprinted paternal homolog, the preeclampsia phenotype was not expressed.

- The spouses of men who were the product of a pregnancy complicated by preeclampsia are more likely to develop preeclampsia than spouses of men without this history (VanDijk *et al.,* 2005).

- A woman who becomes pregnant by a man whose previous partner had preeclampsia is at higher risk of developing the disorder than if the pregnancy with the previous partner was normotensive.

These data suggest that both maternal and paternal contributions to fetal genes may have a role in defective placentation and subsequent preeclampsia. Several candidate genes, such as the angiotensinogen gene variant (T235), endothelial nitric oxide synthase (eNOS), and genes causing thrombophilia, have been linked with preeclampsia, but large studies have not shown them to be important for susceptibility to the disease (Lachmeijer *et al.,* 2002).

Genome wide scanning of 343 Icelandic women with preeclampsia, eclampsia, and gestational hypertension revealed a significant locus at 2p13. Other susceptibility loci have been identified at 2p12, 2p25, and 9p13 (Laivuori *et al.,* 2003). The genes for sFlt-

1 and Flt-1 are carried on chromosome 13. Fetuses with an extra copy of this chromosome (eg, trisomy 13) should produce more of these gene products than their normal counterparts. In fact, the incidence of preeclampsia in mothers who carry fetuses with trisomy 13 is greatly increased compared to all other trisomies or to control pregnant patients. In addition, the ratio of circulating sFlt-1 to PlGF is significantly increased in these women, thus accounting for their increased risk of preeclampsia (Bdolah *et al.,* 2006).

A different locus at 12q may be linked to HELLP syndrome, but not preeclampsia without HELLP syndrome, suggesting that genetic factors important in HELLP syndrome (H = hemolysis, EL = elevated liver function tests, LP = low platelets) may be distinct from those in preeclampsia (Lachmeijer *et al.*, 2001). However, discordant findings have also been reported. For example, a study of preeclampsia in twins failed to find a genetic link (Treloar *et al.*, 2001).

2.6.5 Systemic endothelial dysfunction

All of the clinical features of preeclampsia can be explained as clinical responses to generalized endothelial dysfunction (Redman *et al.*, 1999). As an example, hypertension results from disturbed endothelial control of vascular tone, proteinuria and edema are caused by increased vascular permeability, and coagulopathy is the result of abnormal endothelial expression of procoagulants. Headache, seizures, visual symptoms, epigastric pain, and fetal growth restriction are the sequelae of endothelial dysfunction in the vasculature of target organs, such as the brain, liver, kidney, and placenta.

Laboratory evidence supporting generalized endothelial dysfunction in preeclamptic women includes the following:

- Increased concentrations of circulating cellular fibronectin, factor VIII antigen, and thrombomodulin (Friedman *et al.,* 1995).

- Impaired flow-mediated vasodilation (Eric *et al.,* 2010) and impaired acetylcholine mediated vasorelaxation (Pascoal *et al.,* 1998).

- Decreased production of endothelial-derived vasodilators, such as nitric oxide and prostacyclin, and increased production of vasoconstrictors, such as endothelins and thromboxanes.

- Enhanced vascular reactivity to angiotensin II (Granger *et al.,* 2001).

- Serum from preeclamptic women causes endothelial activation in human umbilical vein endothelial cell culture studies in some in vitro studies (Roberts *et al.,* 1992).

- Impaired endothelial function can be demonstrated by brachial artery flow-mediated dilation three years after a preeclamptic pregnancy (Chambers *et al.,* 2001). It is unknown whether this is a cause or effect of the preeclamptic pregnancy.

The relationship between preexisting vascular disease and susceptibility to developing preeclampsia may be due to preexisting endothelial cell damage (Levine *et al.,* 2006). Preexisting endothelial damage may also explain why women who develop preeclampsia are also at increased risk of developing cardiovascular disease later in life (Bellamy, 2007). Women with a history of preeclampsia are also at increased risk for end stage renal disease and hypothyroidism in the long term (Levine *et al.,* 2009).

Pathogenesis of systemic endothelial dysfunction — Mammalian placentation requires extensive angiogenesis for the establishment of a suitable vascular network to

supply oxygen and nutrients to the fetus. A variety of proangiogenic (VEGF, PlGF) and antiangiogenic factors (sFlt-1) are elaborated by the developing placenta, and the balance among these factors is important for normal placental development. Increased production of antiangiogenic factors disturbs this balance and results in the systemic endothelial dysfunction characteristic of preeclampsia.

sFlt-1, VEGF, PIGF — Soluble fms–like tyrosine kinase 1 (sFlt-1 or sVEGFR-1) is a naturally occurring, circulating antagonist to vascular endothelial growth factor (VEGF). VEGF is an endothelial specific mitogen that has a key role in promoting angiogenesis (Dvorak, 2002). Its activities are mediated primarily by interaction with two high-affinity receptor tyrosine kinases, VEGFR-1 (VEGF receptor-1 or fms-like tyrosine kinase-1 [Flt-1]) and VEGFR-2 (kinase-insert domain region [KDR]/Flk-1), which are selectively expressed on the vascular endothelial cell surface. VEGFR-1 has two isoforms: a transmembranous isoform and a soluble isoform (sFlt-1 or sVEGFR-1). Placental growth factor (PlGF) is another member of the VEGF family that is made predominantly in the placenta. It also binds to the VEGFR-1 receptor. sFlt-1 antagonizes the proangiogenic biologic activity of circulating VEGF and PlGF by binding to them and preventing their interaction with their endogenous receptors. Increased placental expression and secretion of sFlt-1 appear to play a central role in the pathogenesis of preeclampsia, based on the following observations (Ahmad and Ahmed, 2004):

- sFlt-1 administered to pregnant rats induces albuminuria, hypertension, and the unique renal pathologic changes of glomerular endotheliosis.
- In vitro, removal of sFlt-1 from supernatants of preeclamptic tissue culture restores endothelial function and angiogenesis to normal levels. Conversely,

exogenous administration of VEGF and PlGF reverses the antiangiogenic state induced by excess sFlt-1.

- Compared with normotensive controls, circulating levels of sFlt-1 levels are increased and free VEGF and free PlGF are decreased in preeclamptic women. Studies using banked sera showed that preeclamptic women had decreases in PlGF and VEGF levels well before the onset of clinical disease (Thadhani *et al.*, 2004). As an example:

- A nested case-control study using banked sera to measure serum sFlt-1, as well as PlGF and VEGF, across gestation found that changes in sFlt-1 were predictive of the subsequent development of preeclampsia (Levine *et al*, 2004). sFlt-1 levels increased during pregnancy in all women; however, compared to normotensive controls, women who went on to develop preeclampsia began this increase earlier in gestation (at 21 to 24 weeks versus 33 to 36 weeks) and reached higher levels. A significant difference in the serum sFlt-1 concentration between the two groups was apparent five weeks before the onset of clinical disease. PlGF and VEGF levels fell concurrently with the rise in sFlt-1, which may have been related, in part, to binding by sFlt-1.

- In another study, the concentration of sVEGFR-1 correlated with increasing severity of disease: sVEGFR-1 concentrations were higher in women with severe or early (<34 weeks) preeclampsia than in those with mild or late preeclampsia (Chaiworapongsa et al., 2004). Furthermore, women with preeclampsia had higher sVEGFR-1 levels than women who remained normotensive two to five weeks before onset of clinical disease (Chaiworapongsa *et al.*, 2004).

In the aggregate, these observations suggest a major role for sFlt-1 in the pathogenesis of at least some features of preeclampsia (Widmer *et al.,* 2007). However, the trigger for increased sFlt-1 production by the placenta is unknown. The most likely trigger is placental ischemia (Gibert *et al.,* 2008). In vitro, placental cytotrophoblasts possess a unique property to enhance sFlt-1 production when oxygen availability is reduced (Nagamatsu *et al.,* 2004). The increased expression of hypoxia-inducible transcription factors (HIFs) in preeclamptic placentas is consistent with this hypothesis (Rajakumar *et al.,* 2003). It is not known whether increased sFlt-1 secretion is responsible for the early placental developmental abnormalities characteristic of preeclampsia or a secondary response to placental ischemia caused by some other factor. Genetic factors may also play a role in excess production of sFlt-1 and placental size (eg, multiple gestation) may play a role (Bdolah *et al.,* 2008).

Clinical applications for these observations have not been established. In the future, it is possible that drugs binding to sFlt-1 might prevent and treat preeclampsia. Measurement of serum sFlt-1 is still investigational and its ability to predict development of preeclampsia needs to be determined in prospective, longitudinal studies (Widmer *et al.,* 2007). Measurement of sFlt-1:VEGF (or sFlt-1:PlGF) ratio in urine appears to be the best means of screening women to identify those at high risk of developing preeclampsia, and also for confirming clinical diagnosis of the disorder.

Soluble endoglin — It is likely that synergistic factors elaborated by the placenta other than sFlt-1 also play a role in the pathogenesis of the generalized endothelial dysfunction noted in preeclampsia. Consistent with this hypothesis is the observation that the plasma concentration of sFlt-1 protein needed to produce the preeclampsia phenotype in rats was several fold higher than the levels typically seen in patients with

preeclampsia, and no coagulation or liver function abnormalities were reported in the sFlt-1 treated animals.

Eng is a coreceptor for transforming growth factor (TGF)-beta and is highly expressed on cell membranes of vascular endothelium and syncytiotrophoblasts (Venkatesha *et al.,* 2006). A novel placenta-derived soluble form of Eng, referred to as soluble endoglin (sEng), is an anti-angiogenic protein that appears to be another important mediator of preeclampsia (Venkatesha *et al.,* 2006).

Although the precise relationship of sEng to sFlt-1 is unknown, it appears that both sEng and sFlt-1 contribute to the pathogenesis of the maternal syndrome through separate mechanisms. Several lines of evidence support this hypothesis (Venkatesha *et al.,* 2006):

- sEng is elevated in the sera of preeclamptic women two to three months before the onset of clinical signs of preeclampsia, correlates with disease severity, and falls after delivery. An increased level of sEng accompanied by an increased ratio of sFlt-1:PlGF is most predictive of developing preeclampsia.

- In vivo, sEng increases vascular permeability and induces hypertension. In pregnant rats, as an example, it appears to potentiate the vascular effects of sFlt-1 to induce a severe preeclampsia-like state, including the development of HELLP syndrome and restriction of fetal growth.

- sEng inhibits TGF-beta-1 signaling in endothelial cells and blocks TGF-beta-1 mediated activation of eNOS and vasodilation, suggesting that dysregulated TGF-beta signaling may be involved in the pathogenesis of preeclampsia.

Fetuses of preeclamptic mothers do not have high circulating concentrations of either sEng or sFlt-1 (Staff *et al.,* 2007). This suggests that fetuses do not experience proteinuria or hypertension like their mothers because they are not exposed to high concentrations of antiangiogenic factors.

2.3.6 Inflammation

Circulating syncytiotrophoblast debris have been hypothesized to contribute to maternal inflammation and some of the features of the maternal syndrome (Redman and sargent 2005). Signs of maternal inflammation which appear to be present in normal pregnancies at term are exaggerated in preeclampsia. Trophoblastic debris and the microparticles shed during normal pregnancy are proinflammatory and this process is amplified in preeclampsia (Germain *et al.,* 2007). It is likely that the inflammatory state may increase the vascular endothelial sensitivity to toxic factors such as sFlt1 and sEng, although definitive evidence is lacking.

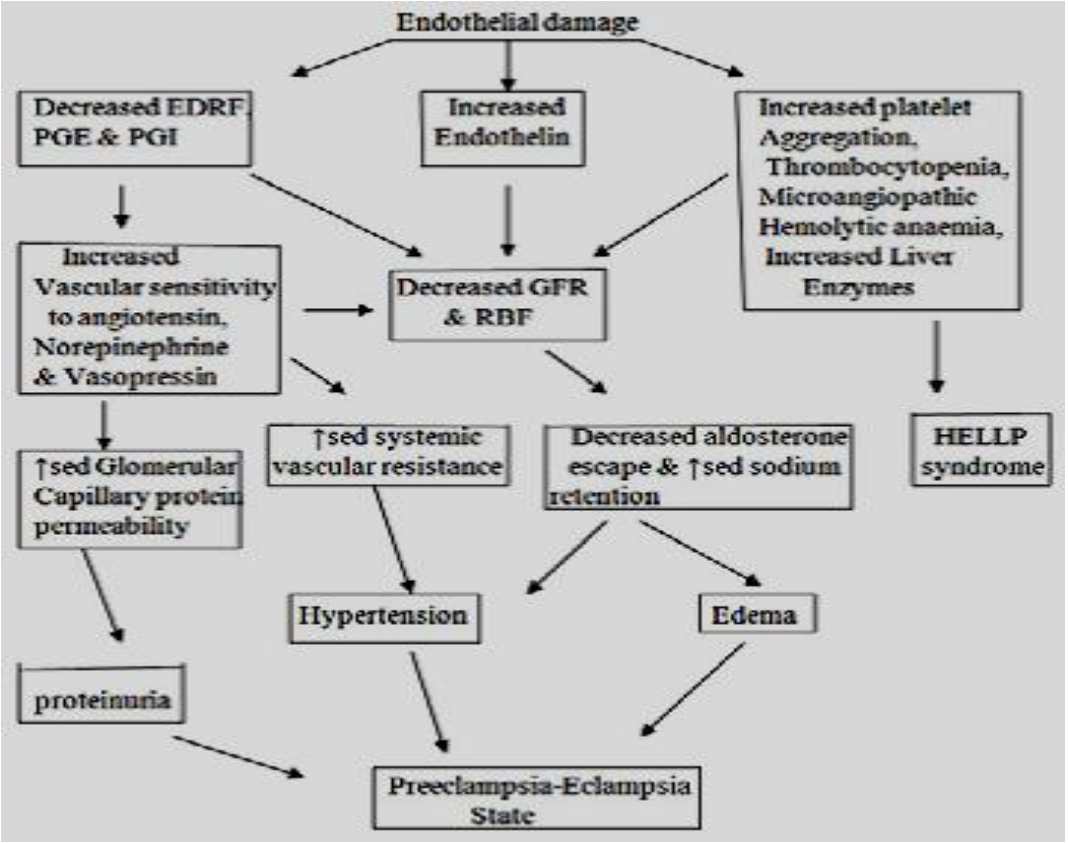


Figure 2.1 Pathophysiologic events that characterize Preeclampsia-Eclampsia (Schrier and Briner 1991).

2.7 Management of Preeclampsia and Eclampsia

Preeclampsia is a common cause of maternal and perinatal morbidity and mortality in both developed and developing countries. Early delivery is the only effective treatment, but necessitates preterm birth in many cases. Thus, an intervention that could prevent preeclampsia would have a significant impact on maternal and infant health worldwide. Many different strategies to prevent preeclampsia have been investigated in randomized controlled trials, but none have been found to be widely effective. It is not surprising that these simple approaches have been unsuccessful, given the complexities in pathogenesis and the likelihood that multiple etiologies cause the syndrome even though the clinical manifestations of elevated blood pressure and proteinuria are common to

most cases.Meticulous antepartum care, including close follow-up of high risk women after mid-gestation, will increase the chance that early signs of preeclampsia will not be overlooked. Thus, although preeclampsia may not be preventable at present, early diagnosis followed by appropriate management may prevent some of the dangerous sequelae of the disease, such as eclamptic seizures and multiorgan organ failure. There is no effective intervention for the general obstetrical population, low dose aspirin appears to be useful in women at high risk of developing preeclampsia (Subtil *et al.,* 2003b).

Antiplatelet Agents — The observation that preeclampsia is associated with increased platelet turnover and increased platelet-derived thromboxane levels led to randomized trials evaluating low-dose aspirin therapy in women thought to be at increased risk of the disease (Dekker and Sibai 1993). As opposed to higher dose aspirin therapy, low-dose aspirin (60 to 150 mg per day) diminishes platelet thromboxane synthesis while maintaining vascular wall prostacyclin synthesis (Dekker and Sibai 1993). The drug has been studied for both prevention of preeclampsia and prevention of progression of mild to severe preeclampsia. It appears to result in a modest reduction in risk of preeclampsia when given to women at moderate to high risk of the disease.

This approach has been studied in over 35,000 women, for both prevention of preeclampsia and prevention of progression of mild to severe preeclampsia. Low dose aspirin has a modest impact on pregnancy outcome; it reduces the risk of preeclampsia, as well as other adverse pregnancy outcomes (e.g, preterm delivery, fetal growth restriction) by about 10 to 15 percent. Low dose aspirin is safe in pregnancy; thus, it is a reasonable strategy in women with a moderate to high risk of preeclampsia.

Primary prevention of preeclampsia

Initial studies of highest risk women — Early studies assessing the efficacy of low-dose aspirin therapy were performed in women at especially high risk for developing preeclampsia. Entry into these studies required risk factors such as a positive angiotensin II infusion test early in the third trimester (Wallenburg, 1989), history of severe early-onset preeclampsia with fetal growth restriction or death (Benigni *et al.*, 1989), or historical risk factors plus a positive roll over test (Schiff *et al.*, 1989).

These small studies (33 to 102 patients) suggested that low-dose aspirin was an effective therapy in this subgroup of women; the incidence of preeclampsia was only 0 to 3 percent in treated patients versus 12 to 35 percent in controls receiving no treatment or a placebo (Schiff *et al.*, 1989). Additionally, low-dose aspirin therapy generally led to an absolute decrease in thromboxane production, relatively little change in prostacyclin or prostaglandin E synthesis, and therefore a decrease in the ratio of thromboxane to prostacyclin. By comparison, placebo therapy was associated with a progressive increase in the thromboxane to prostacyclin ratio (Dekker and Sibai 1993). Some treated women, however, had an equivalent reduction in thromboxane and prostacyclin levels with no improvement in the ratio; these women had a high rate of pregnancy-induced hypertension and did not appear to benefit from low-dose aspirin therapy (Brown *et al.*, 1990).

Results from large trials of at risk women — In contrast to the above findings from initial small studies in selected women at very high risk, subsequent large trials including both moderate and high risk women did not show a significant reduction in the incidence of preeclampsia with low-dose aspirin therapy. The following examples illustrate the range of findings in the largest of these trials:

- An Italian trial randomly assigned over 1100 patients to either aspirin (50 mg) or no therapy at 16 to 32 weeks. Criteria for inclusion included age under 18 or over 40 years, mild or moderate chronic hypertension, or history of pregnancy-induced hypertension.

- The National Institute of Child Health and Human Development Network of Maternal-Fetal Medicine Units double-blind study randomly assigned 2539 women to low-dose aspirin (60 mg) or placebo at 13 to 26 weeks of gestation (Caritis, *et al.*, 1998). Criteria for inclusion included the presence of one of the following high-risk conditions: pregestational insulin-treated diabetes mellitus, chronic hypertension, multifetal gestation, or a previous history of preeclampsia. There was no significant reduction in the incidence of preeclampsia with active therapy (18 versus 20 percent in controls) overall or in any of the high-risk subgroups. In the aspirin and control groups, the incidence of preeclampsia was 18 and 22 percent, respectively, among women with pregestational diabetes; 26 and 25 percent, respectively, in women with chronic hypertension; 12 and 16 percent, respectively, in multifetal gestations; and 17 and 19 percent, respectively, in women with preeclampsia in a previous pregnancy.

Low-dose aspirin therapy in nulliparous patients may modestly decrease the risk of preeclampsia (Sibai *et al.,* 1993), but does not affect birth weight, the incidence of fetal growth restriction, or length of gestation (Subtil *et al.,* 2003b). One hypothesis for the lack of a significant effect may be that even though the incidence of preeclampsia in unselected nulliparous women is higher than in multiparas (incidence 2 to 6 versus 1 percent), it is still relatively low compared to women with risk factors such as chronic hypertension (incidence 10 percent) or renal disease (incidence 20 percent). It is also possible that the pathogenesis of preeclampsia in nulliparous women is different from

that in women with previous preeclampsia or preexisting vascular disease (Sibai *et al.*, 2005).

Women with abnormal uterine artery Doppler — Abnormal uterine artery Doppler velocimetry may be the best clinically available tool for identifying women at high risk of developing preeclampsia; however, the clinical value of routine Doppler surveillance has not been proven.

A French trial randomly assigned 1253 nulliparous women to uterine artery Doppler surveillance at 23 to 24 weeks of gestation with aspirin therapy (100 mg/day) if the Doppler was abnormal and 617 women to no Doppler studies and placebo treatment (Subtil *et al.*, 2003a). Abnormal uterine artery Doppler studies were identified in 239 women. The major findings from this trial were:

- Abnormal uterine artery Doppler studies identified women who were likely to develop preeclampsia and fetal growth restriction (preeclampsia 6 versus 1 percent in abnormal and normal Doppler groups, respectively; growth restriction 18 versus 8 percent).

- Doppler surveillance with low-dose aspirin treatment of the 20 percent of women with abnormal results did not reduce the incidence of preeclampsia compared to no surveillance. Preeclampsia occurred in approximately 2 percent of patients in each group. Treatment also did not reduce the incidence of fetal growth restriction.

This trial confirmed the predictive value of uterine artery Doppler, but failed to demonstrate the value of routine screening followed by low-dose aspirin therapy for a positive test compared to routine prenatal care. Possible limitations of this trial are that low-dose aspirin therapy was started relatively late in pregnancy (22 to 25 weeks), after

significant pathologic changes had already occurred in the uteroplacental vasculature, and the low rate of preeclampsia may have precluded finding a significant reduction in disease.

Approach to therapy — Major questions remain as to whether any subgroup of women is likely to benefit from low-dose aspirin therapy, when treatment should be started (first or second trimester), and the optimum dose to inhibit placental PGH synthase (cyclooxygenase) and also allow the anti-inflammatory effects of low-dose aspirin (Dekker and Sibai 2001).

The American College of Obstetricians and Gynecologists practice bulletin does not recommend low-dose aspirin therapy for women at low-risk for development of preeclampsia (ACOG, 2002). However, no conclusions were reached about moderate to high risk women.

A Cochrane review concluded that for moderate and high risk women, 59 to 167 patients would have to be treated to prevent one case of preeclampsia; 44 to 200 would have to be treated to prevent one preterm birth; and 125 to over 10,000 would have to be treated to prevent one perinatal death (Knight *et al.,* 2000).The NICE guideline on management of hypertensive disorders during pregnancy advises use of low dose (75 mg) aspirin for women with at least one high risk factor for preeclampsia (chronic hypertension or kidney disease, diabetes, autoimmune disease, hypertension in previous pregnancy) or at least two moderate risk factors for preeclampsia (age ≥40 years, first pregnancy, multiple gestation, >10 years between pregnancies, body mass index ≥35 kg/m2 at presentation, family history of preeclampsia) (Visintin *et al.,* 2010).

Some perinatologists recommend low-dose aspirin for women at moderate or high risk for preeclampsia, as there is a potential for benefit with minimal risk. There is no

consensus on the criteria that confer significant risk; previous pregnancy with early preeclampsia and a poor fetal outcome, type 1 diabetes mellitus with vascular involvement, antiphospholipid syndrome, and poorly controlled chronic hypertension are the most common indications.

Treatment should be begun at 12 to 14 weeks of gestation (Visintin *et al.,* 2010). The optimum low dose is unclear; 81 mg is readily available. The safety of low-dose aspirin use in the second and third trimesters is well-established (Coomarasamy *et al.,* 2003), but questions linger regarding use in the first trimester. Early therapy is probably important since the pathophysiologic features of preeclampsia develop at this time, weeks before clinical disease is apparent. Aspirin is discontinued 5 to 10 days before expected delivery to diminish the risk of bleeding during delivery (Hirsh *et al.,* 2008); however, no adverse maternal or fetal effects related to low-dose aspirin have been proven.

Prevention of worsening preeclampsia — another setting in which low-dose aspirin appears to be of little or no benefit is in women who already have developed pregnancy-induced hypertension (CLASP, 1994). At this late stage, aspirin does not prevent progression to more severe disease and may exacerbate a bleeding diathesis in patients with the HELLP syndrome.

Anticoagulation — the preeclamptic placenta displays characteristic features of uteroplacental ischemia, including increased syncytial knots and intervillous fibrin, distal villous hypoplasia, villous infarcts, decidual necrosis, and spiral artery abnormalities including acute atherosis, mural hypertrophy, and luminal thrombosis/fibrous obliteration. Prophylactic anticoagulation in selected high risk women, particularly those with previous early severe preeclampsia, has been suggested

to minimize formation of thrombi and maintain perfusion, although thrombus formation is a minor component of these uteroplacental abnormalities (Rey *et al.*, 2009). This area remains controversial.

Calcium Supplementation — as with aspirin, there does not appear to be any benefit to routine calcium supplementation for healthy, nulliparous women in whom baseline dietary calcium intake is adequate. There may be a benefit for preeclampsia prevention in some high-risk populations, particularly those who eat a low calcium diet (Hofmeyr, 2010).

Calcium supplementation (1.5 to 2 g of calcium carbonate per day in divided doses) has been evaluated for prevention of hypertensive disorders of pregnancy. The potential value of this approach was illustrated in a systematic review of 13 randomized trials including over 15,000 women that assessed the effects of calcium supplementation on development of pregnancy-related hypertensive disorders (Hofmeyr, 2010). Calcium supplementation appeared to approximately halve the risk of preeclampsia (RR 0.45, 95% CI 0.31-0.65), to reduce the risk of preterm birth (RR 0.76, 95% CI 0.60-0.97), and to reduce the rare occurrence of the composite outcome "maternal death or serious morbidity" (RR 0.80, 95% CI 0.65-0.97). There were no other clear benefits, or harms. The reduction in risk ratio was greatest for women at high risk of preeclampsia (five trials, 587 women: RR 0.22, 95% CI 0.12-0.42), and for those with low baseline calcium intake (eight trials, 10,678 women: RR 0.36, 95% CI 0.20-0.65). The largest trial of women with low baseline calcium intake did not show a substantial reduction in preeclampsia, but there was a significant reduction in early onset/severe disease (Villar *et al.*, 2006).

Antioxidants — the pathogenesis of preeclampsia has been described as a two-stage process: reduced placental perfusion followed by the release of placental factors that trigger maternal endothelial cell dysfunction (Roberts, 1999). Oxidative stress may be a cause of this endothelial cell dysfunction. This hypothesis is supported by the observation that markers of oxidative stress are present in the maternal circulation of affected women (Roberts and Speer 2004). For this reason, antioxidants have been proposed for prevention and treatment of preeclampsia. Antioxidants also have other effects that may or may not be detrimental to pregnancy (Banerjee *et al.*, 2006).

Other dietary interventions — there are only a few small randomized trials evaluating the role of diet in prevention of preeclampsia. No beneficial results have been demonstrated from these interventions, which include nutritional advice, protein and energy supplements, protein and energy restriction (in obese women), magnesium supplementation, and salt restriction (Villar *et al.*, 2004). Vitamin D supplements — Some observational studies have reported an association between vitamin D deficiency and an increased risk of preeclampsia (Bodnar *et al.*, 2007a, 2007b) and early onset severe preeclampsia (Robinson *et al.*, 2010), but a prospective cohort study of women at high risk for preeclampsia did not find an association (Shand *et al.*, 2010).

Antihypertensive Drugs — Controlled studies have not demonstrated that lowering the blood pressure with antihypertensive medications (including diuretics (Churchill *et al.*, 2007)) reduces the risk of preeclampsia or abruption, or improves fetal or maternal outcome; however, therapy does decrease the incidence of severe hypertension.

Nitric Oxide — Preeclamptic women may be deficient in nitric oxide, which mediates vasodilatation and inhibits platelet aggregation. However, there is no high quality

evidence that administration of nitric oxide donors (glyceryl trinitrate) or precursors (L-arginine) prevents preeclampsia (Meher and Duley 2007).

2.8 Current service provision

Screening for women at risk of pre-eclampsia is an important part of antenatal care. Routine screening for pre-eclampsia is based on measurement of blood pressure and urinalysis for proteinuria. Once women have been identified as been at high risk, they can be targeted for more intensive antenatal surveillance and prophylactic interventions such as early delivery. Most current strategies for risk assessment are based on the obstetric and medical history and clinical examination. Pregnant women are assessed at their first antenatal clinic (prior to 12 weeks if possible) for risk factors for pre-eclampsia, including age, nulliparity, long pregnancy interval, prior history of pre-eclampsia, high body mass index, history of diabetes mellitus and hypertension. If a woman has any of the risk factors, an increased schedule of blood pressure screening is provided. Otherwise, they have blood pressure measurement and urinalysis for proteinuria at 16, 25, 28, 31, 34, 36, 38 and 40 weeks. No other blood tests to detect pre-eclampsia are recommended and routine Doppler ultrasound scans of the uterine or umbilical artery are not recommended (Anon, 2003).

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

3.1.1 Chemicals and Reagents

ELISA kit (Abnova chemical Company, USA) ,Biuret reagent: stock, 0.2N Alkaline iodide, Standard of protein, Succinate buffer (0.1M, pH 4),Dye stock – 0.06 (10mmol/l),Working BCG pH 4.15 at 20^0C 100ml succinate buffer,8.0ml stock BCG,2.50ml Triton x 100, 2.50ml stock azide and made up to 1 litre with distilled water and adjusted to pH 4.10

3.1.2 Equipment

Incubator, stop watch, micropipette, Electrolyte Analyser (Audicom AC9900) instrument, metler balance, incubator, UV-Visible spectrophotometer (6405 Jenway UV-Visible spectrophotometer), pH meter (Jenway 3159), Seca weighing scale and heightometer, centrifuge (Labofuge 300) and Atomic Absorption Spectrophotometer (AS 240 FS Varian). Most of the equipment used werelocated in the Departments of Obstetrics and Gynaecology, Heamatology, Multipurpose Laboratory and Medical laboratory of University Health Services (Sickbay), Ahmadu Bello University, Zaria-Nigeria.

3.1.3 Questionnaire

Semi-structured pre-tested questionnaire was used to capture the socioeconomic status, Blood pressure, Body mass index and dietary intake of respondents.

3.2 Methods

3.2.1 Study Area

Ahmadu Bello University Teaching Hospital, Shika, Sabon Gari local Government Area of Kaduna State, Nigeria was used for the study, because it is patronized nationwide

3.2.2 Sample size

The sample size used in this study was calculated using the formula for case- control study (Lischko *et al.*, 1989).

Thus:

$$n = (\frac{r+1}{r}) \frac{\sigma^2 (Z_\beta + Z_{\alpha/2})^2}{(difference)^2}$$

Assuming

80% power, Z_β=.84, 0.05 significance level, Z_α=1.96, r=1 (equal number of cases and controls), σ=5.0, Difference = 2.5

Sample Size = 63 (30 cases and 30 controls)

3.2.3 Sampling technique

A consecutive sampling technique was used to get pregnant patients who attend the Department of Obstetrics and Gynaecology Clinic in the Hospital until the sample size was saturated.

3.2.4 Ethical approval

The Ethical and Research Committee of the Ahmadu Bello University Teaching Hospital, Zaria, approved the study protocol with approval number

ABUTH/HREC/TRG/36 and informed consent was obtained from the controls and the patients before enlistment into the study and collection of the blood samples.

3.2.5 Inclusion and Exclusion criteria

Inclusion criteria: Clinically diagnose pre-eclampsia and eclampsia patients in their second trimester with significant minimum proteinuria of +2 and Patients with elevated blood pressure.

Exclusion criteria: Patients that are very ill or unconscious and patients with non-proteinuric Pre-eclampsia and eclampsia.

3.2.6 Study grouping and experimental design

A total of sixty (60) subjects were recruited for the study. The subjects were divided into four groups:

Control group I –Fifteen (15) women having normal pregnancy without hypertension;

Control group II –Fifteen (15) pregnant women with non proteinuric hypertension;

Case Group III –Twenty Five (25) pregnant women having Proteinuric preeclampsia;

Case Group IV –Five (5) pregnant women with eclampsia.

3.3.1 Blood pressure measurement

Both systolic and diastolic blood pressures were measured with a standard digital sphygmomanometer and standard arm cuff at the right arm. The recordings were taken at interval of 5 minutes each, and the readings recorded for systolic and diastolic blood pressure respectively (Guido *et al.,* 1998). The degree of hypertension was directly

determined from the blood pressure measured. Women once found hypertensive, were screened for presence of protein in urine by using the dipstick method.

3.3.2 Measurement of mean arterial pressure

Mean Arterial Pressure was calculated as = 1/3 (SBP) + 2/3 (DSP) (Howard *et al.*, 2000)

SBP-Systolic Blood Pressure, DSP-Diastolic Blood Pressure

3.4 Determination of dietary intake

Dietary recall was achieved through administration of a semi structured pretested questionnaire and 24- hour food recall kit that were subsequently used to estimate the dietary intake of subjects (FAO, 2012).

3.5 Sample collection and preparation

Five milliliters of venous blood was collected from the antecubital vein. The blood was allowed to clot and the serum was separated by centrifugation. Haematological parameters were assessed soon after the collection but other tests were conducted within four (4) to Six (6) hours after collection.

3.6 Assay of Biochemical Parameters

3.6.1 Serum electrolyte assay by Ion selective electrode.

Principle: This was done using Audicom (AC9900) electrolyte analyzer. Basically the ion selective electrode generates a difference in potential between itself and a reference electrode, the output potential is proportional to the amount or concentration of the selected ion in solution. (Tietz, 1990).

Procedure: The auto sampler holder containing 20 samples, rotate towards the capillary inlet where 160ul of the sample is aspirated into the analyzer, the already calibrated machine analyse Potassium, Sodium, Chlorine, Biocarbonate and calculate serum non-ionised calcium and calcium. The results were displayed on the screen of the analyser.

3.6.2 Estimation of serum magnesium

Principle: Serum magnesium concentration was estimated by using Atomic Absorption spectrophotometer (AA 240FS Varian, Sweden).In the AAS method, light of the desired wavelength is passed through the flame, which contains the atomized analyte. The amount of light absorbed by the analyte depends upon the concentration of analyte (Beer's law). By measuring the amount of light absorbed by the flame, it is possible to determine the concentration of analyte in the sample.

Procedure: The serum magnesium levels was estimated by diluting 5µl of the serum with 50ml of distilled water, the mixture was shaken and from the mixture 5ml was placed into the spectrophotometer.

3.6.3 Assessment of parathyroid hormone

The serum parathyroid hormone was assessed using a two-site ELISA (Enzyme-Linked Immunosorbent Assay).

Procedure: Sufficient streptavidin coated strips in a holder were used to run all six (6) PTH calibrators, A-F of the intact PTH. Then Pipetting 25µl of sample into the designated well followed by addition of 50µl of biotinylated and enzyme labeled antibody into each of the wells containing the samples. The microplate covered with aluminium foil was placed on a shaker at 180 rpm for 3 hours 30 minutes at room

temperature. The fluid was then aspirated completely and then each well washed five (5) times with the working wash solution by dispensing 0.35 ml into each well.

Then 150µl of the TMB (tetramethylbenzidine) substrate was dispensed into each of the wells with appropriate cover to avoid light exposure, the microplate was placed on a shaker 180 rpm for 30 minutes at room temperature after which 100 µl of stop solution is dispense into each of the wells and mix gently. The absorbance of the solution in the wells was taken within 15 minutes, using a microplate reader (Hollborn ELISA reader 73A) set to 450 nm and 405 against 250µl of distilled water respectively.

Calculation: The concentration of PTH was calculated using masterplex readerfit ELISA software.

3.6.4 Estimation of protein concentration

Principle: The protein concentration in the serum was quantified by Biuret method (1985).Bovine serum Albumin (4mg/dl) was used as standard. The copper ions in the biuret reagent complexes with the peptide linkage in proteins in an alkaline environment form a violet colour complex which absorbs maximally at 540nm. The intensity of the violet coloured-complex formed is directly proportional to the quantity of protein present in the sample..

Procedure: To 0.09ml of the serum was added 4ml of Biuret reagent and incubated at 37^0C for 10 minutes and their absorbance read at 540nm in a spectrophotometer

Calculation:

$$\text{Serum total protein (g/L)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{Concentration of standard}$$

3.6.5 Estimation of serum albumin concentration

Principle: Albumin concentration was estimated using modification of Bartholomew and Delaney method, (1966). Albumin reacts with the dye-Bromocresol green which has specific affinity for albumin in acidic solution when they react to produce a coloured complex which is measured calorimetrically. The intensity of this colour produced is proportional to the concentration of albumin in serum sample.

Procedure: Serum (0.05ml) was added to 4.00ml working colour. Solution mixed and incubated at 37^0C for 10 minutes. The absorbance was then read at 628nm using BCG to zero the spectrophotometer.

Calculation

$$\text{Serum Albumin (g/100ml)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{Concentration of standard}$$

Where concentration of standard = 30g/L

3.6.6 Evaluation of haematological parameters

The haematology analyser (Swelab Alpha Autohaemtoanalyser)is based on the principle of impedance measurement (Coulter principle). An electrical current flow is established through an aperture of known dimension. The cell particles impede the current flow causing a voltage surge (resistance during cell passage through a small defined opening between two electrodes). The voltage, first rises and then falls below a threshold, indicating the passage of a particle through the sensing zone. Cells and particles are counted by monitoring the number of voltage pulses that occur during a specified measurement interval (Chapman, 2000).

Procedure: The blood sample was lifted on to the micro capillary inlet- which allows a 20µl of the sample to be aspirated directly in the system and diluted with an isotonic saline solution. The analyser prepares two dilutions: lysed and unlysed. After the first solution is measured, the white blood cell (WBC) and Hemoglobin (Hb) values were taken; the analyzer processes the second dilution, which measures Red blood cell (RBC), Haematocrit (HCT), platelet (PLT) and others. The result were displayed on the screen of the analyser.

3.7 Statistical Analysis

The data obtained were expressed as Mean ± Standard deviation using SPSS (version 20.0). Data were analyzed by One-way ANOVA at ($p<0.05$) and difference between means was separated by Duncan multiple range test.

3.8 Study Limitation

Due to non-availability of assay kit within the country and the financial burden of sending the samples abroad, serum Vitamin D could not be determined

CHAPTER 4

RESULTS

4.1 Socio-Economic/Demographic Characteristics and Blood Pressure of Pregnant Women Normotensive, Hypertensive, proteinuric pre-eclamptic and eclamptic (N= 60)

The socioeconomic status of women normotensive, hypertensive and those with proteinuric preeclampsia are each summarised in Table 4.1. Most of the women that participated in the study are within the age range of 26-30 years with the highest in the women with preeclampsia (60%) and percentage age of ≤20 low in all the study group. Their age at marriage range of 21-30 was highest in the normotensive women (66.7%) and none of the respondent is above fifty years. Most of the subjects were Hausa women (80%), none of the normotensive pregnant women and eclamptic pregnant women was Igbo and they all have formal education with majority having tertiary education (86.7%) and 4% of the preeclamptic pregnant women with Islamiyya education . Majority of the subjects live on less than ₦5,000 as their monthly income and only 6.7% of the normotensive pregnant women live on monthly income above ≥₦55,000. Although majority (40%) of the normotensive and hypertensive women are civil servants, 40% of the eclamptic pregnant women are housewives.

The result of the blood pressure of the subjects is showed in Table 4.2 indicate gradual increase in the systolic and diastolic blood pressure of the respondents which was reflected in the mean arterial pressure of all the study subjects with highest in the women with eclampsia (131.34 ± 6.07) and lowest (88.34 ± 12.59) in the normotensive pregnant women.

Table 4.1 Socio-Economic/Demographic Characteristics of Pregnant Case and Control (N = 60)

Parameters	Study Groups (%)			
	Normotensive (n =15)	Hypertensive (n =15)	Preeclamptic (n =25)	Eclamptic (n =5)
Age (yrs)				
≤20	0	6.7	8.0	0
21-25	26.7	26.7	12.0	20.0
26-30	40.0	33.3	36.0	60.0
31-35	6.7	33.3	24.0	20.0
>35	26.7	0	20.0	0
Age at marriage	**(yrs)**			
≤20	36.7	46.7	44	60
21-30	66.7	40.0	52	40
31-40	6.7	13.3	0	0
41-50	0	0	4	0
>50	0	0	0	0
Ethnicity				
Hausa	53.3	60.0	64.0	80.0
Yoruba	13.3	6.7	12.0	0
Igbo	0	13.3	16.0	0
Others	33.3	20.0	8.0	20.0
Educational Status				
Islamiyya	0	0	4.0	0
Primary	0	6.7	20.0	40.0
Secondary	13.3	20.0	28.0	20.0
Tertiary	86.7	73.3	48.0	40.0
No Formal educ.	0	0	0	0
Occupation				
Civil Servant	40.0	40.0	20.0	20.0
Trader	6.7	13.3	32.0	20.0
Housewife	26.7	33.3	28.0	40.0
Farmers	0	0	0	0
Others (Artisan)	26.7	13.3	20.0	20.0
Monthly income				
<₦5,000	40.0	46.7	64.0	80.0
₦5,000-₦14,999	0	20.0	4.0	0
₦15,000-₦24,999	6.7	6.7	4.0	20.0
₦25,000-₦34,999	26.7	13.3	20.0	0
₦35,000-₦44,999	13.3	6.7	8.0	0
₦45,000-₦54,999	6.7	6.7	0	0
≥₦55,000	6.7	0	0	0

Table 4.2 Blood Pressure of Pregnant Women Normotensive, Hypertensive, proteinuric pre-eclamptic and eclamptic (N= 60)

Blood Pressure (mmHg)	Study Groups			
	Normotensive (n =15)	**Hypertensive (n =15)**	**Preeclamptic (n =25)**	**Eclamptic (n =5)**
Systolic	118.00 ± 8.19^{a}	152.70 ± 15.78^{b}	160.40 ± 12.32^{b}	182.00 ± 8.36^{c}
Diastolic	77.67 ± 9.79^{a}	90.87 ± 7.68^{b}	99.20 ± 7.99^{c}	106.00 ± 5.47^{c}
Mean Arterial Pressure	88.34 ± 12.59^{a}	109.20 ± 10.54^{b}	121.34 ± 8.02^{bc}	131.34 ± 6.07^{C}

Values are means ± SD. Values with different superscript denote significant difference along the rows ($P < 0.05$)

4.2 Dietary Intake of Pregnant Women Normotensive, Hypertensive, proteinuric pre-eclamptic and eclamptic (N= 60)

Macronutrient, micronutrient and mineral intake of women normotensive, hypertensive, proteinuric preeclampsia and those with eclampsia is summarised in Tables 4.3 and 4.4 respectively. The mean energy intake of subjects was lowest in the eclamptic pregnant women (858.00±195.16) and highest in the hypertensive pregnant women (2303.20±2048.05), calcium intake was lowest in normotensive pregnant women (354.32±97.96) and highest in eclamptic pregnant women (500.50±253.85), however there was no significant difference ($P > 0.05$) in the levels of intake of macronutrient, micronutrient and mineral intake of all the subjects but the dietary intake of Magnesium, Potassium and vitamin E of all the subjects were below the recommended dietary allowance (RDA) for pregnant women except in the case of the hypertensive pregnant women that have higher intake of magnesium compared to the RDA.

4.3 Serum Electrolytes and Parathyroid Hormone Levels of Pregnant Women Normotensive, Hypertensive, proteinuric pre-eclamptic and eclamptic (N= 60)

The levels of some electrolytes, pH and Parathyriod Hormone of pregnant normotensive, hypertensive and proteinuric preeclampsia and eclampsia subjects are presented in Table 4.5. It was observed that serum sodium was lowest in preeclamptic patients (135.9±1.07) and highest in the hypertensive pregnant women (158.20±19.20), serum chloride was lowest in the normotensive pregnant women (98.50±0.58) and highest in the hypertensive pregnant women (110.50±10.40) and serum potassium values ranges from 3.77 mmol/L to 4.43 mmol/L in the eclamptic and preeclamptic pregnant women respectively. There was no significant difference ($P > 0.05$) in all the study groups; however there was significant

difference ($P < 0.05$) in the levels of Bicarbonate between the normotensive subjects (32.10±2.22) and hypertensive subjects (17.30±1.56). However the blood pH for all the study subjects were higher than normal except for that of the hypertensive subjects (7.44±0.05). There was significant decrease in the levels of total, ionised and non-ionised calcium in all the study subjects while values of serum magnesium increased significantly ($p<0.05$) in all the study groups except for the normotensive pregnant subjects (1.93± 0.13). There was no significant difference ($p > 0.05$) in the levels of serum parathyroid hormone in the normotensive (21.96±0.46) and hypertensive subjects (24.02±0.69) and the preeclamptic (29.46±1.20) and eclamptic subjects (29.16±4.53).

4.4 Serum Protein and some Heamtological parameters of Pregnant Women Normotensive, Hypertensive, proteinuric pre-eclamptic and eclamptic (N= 60)

The levels of serum proteins in women with preeclampsia and eclampsia is shown in Table 4.6 The normotensive women have the lowest levels of serum total protein 5.16 ± 0.34 while those with preeclampsia have the highest amount of total protein 6.21± 0.45. Serum albumin was statistically not significant ($p>0.05$)in the eclampsia subjects 3.25 ± 0.36 compared to the hypertensive pregnant women with serum albumin of 4.11±0.24. There is no significant difference in the levels of serum albumin among the study groups.

Table 4.3 Mean Dietary Macronutrient Intakes of Pregnant Women Normotensive, Hypertensive, proteinuric pre-eclamptic and eclamptic (N= 60)

Parameters	Study Groups				
	Normotensive (n =15)	**Hypertensive (n =15)**	**Preeclamptic (n =25)**	**Eclamptic (n =5)**	**RDA**
Energy (kcal)	1689.60±526.59[a]	2303.20±2048.05[a]	930.25±204.10[a]	858.00±195.16[a]	2500
Water (g)	475.17±148.5[a]	872.56±734.33[a]	267.07±177.8[a]	560.50±96.87[a]	2.5
Protein (g)	80.65±36.90[a]	260.68±222.58[a]	165.57±92.07[a]	100.00±43.27[a]	71
Fat (g)	225.85±139.45[a]	412.00±370.24[a]	270.25±130.1[a]	400.50±28.99[a]	70
Carbohydrate(g)	168.97±24.24[a]	207.60±178.07[a]	506.50±140.2[a]	123.20±18.66[b]	175
Dietary Fiber(g)	57.10±15.65[a]	69.44±59.06[a]	50.47±35.61[a]	27.50±15.13[a]	28

Values are means ± SD from dietary recall. Values with different superscript denote significant difference along the rows ($P < 0.05$)

Table 4.4 Mean Dietary Micronurient and Mineral Intakes of Pregnant Women Normotensive, Hypertensive, proteinuric pre-eclamptic and eclamptic (N= 60)

Parameters	Study Groups				
	Normotensive (n =15)	**Hypertensive (n =15)**	**Preeclamptic (n =25)**	**Eclamptic (n =5)**	**RDA**
Calcium (mg)	354.32±97.96 [a]	505.96±433.16[a]	339.75±131.03 [a]	500.50±253.85[a]	1300
Iron (mg)	151.02±32.94 [a]	226.56±191.86[a]	281.25±125.36[a]	332.00±164.04[a]	27
Magnesium (mg)	233.00±150.27[a]	421.20±371.54 [a]	329.00±228.87 [a]	258.50±88.38[a]	400
Potassium (mg)	870.60±184.52[a]	1008.12±927.96[a]	900.50±172.56[a]	819.00±213.54[a]	4700
Sodium (mg)	54167.50±17183 [a]	928.00±790.34 [a]	297.05±272.46 [a]	176.00±9.05 [b]	1500
Vitamin E (mg)	3.80±0.91[a]	4.87±4.16[a]	3.40±0.71[a]	3.63±0.68[a]	15
Vitamin C (mg)	190.67±62.67[a]	227.22±195.10[a]	268.00±115.49 [a]	350.00±98.99 [a]	85

Values are means ± SD from dietary recall. Values with different superscript denote significant difference along the rows ($P < 0.05$)

Table 4.5 Serum Electrolytes and Parathyroid Hormone Levels of Pregnant Women Normotensive, Hypertensive, proteinuric pre-eclamptic and eclamptic (N= 60)

Parameters(mmol/L)	Study Groups			
	Normotensive (n =15)	**Hypertensive (n =15)**	**Preeclamptic (n =25)**	**Eclamptic (n =5)**
Na^+	136.40±0.66[a]	158.20±19.20[a]	135.9±1.07[a]	144.30±1.7[a]
Cl^-	98.50±0.58[a]	110.50±10.40[a]	102.40±0.55[a]	98.70±1.83[a]
K^+	3.89±0.11[a]	4.02±0.99[a]	4.34±0.27[a]	3.77±0.81[a]
HCO_3^-	32.10±2.22[a]	17.30±1.56[b]	35.70±2.32[a]	37.20±0.82[a]
pH	7.52±0.04[a]	7.44±0.05[a]	8.17±0.09[b]	8.16±0.14[b]
TCa	2.44±0.02[a]	2.18±0.05[b]	2.23±0.07[b]	1.25±0.51[b]
iCa	1.18±0.01[a]	1.06±0.03[b]	0.91±0.05[bc]	0.43±0.25[c]
nCa	1.24±0.01[a]	1.09±0.01[b]	1.32±0.03[bc]	0.82±0.26[c]
Mg^+	1.93±0.13[a]	1.99±0.10[a]	3.22±0.69[ab]	4.66±0.80[b]
Parathyroid Hormone	21.96±0.46[a]	24.02±0.69[a]	29.46±1.20[b]	29.16±4.53[b]

Values are means ± SD. Values with different superscript denote significant difference along the rows ($P < 0.05$) where TCa-Total calcium, iCa-Ionised calcium, nCa- non-ionized calcium.

Results of haematological parameters are summarised in Table 4.5. The PCV levels were lowest (32.53±1.54) in the hypertensive pregnant women while that of the preeclamptic pregnant women was highest (40.40±3.66) and significantly different ($P < 0.05$) from the other subjects ($p > 0.05$). White blood cells was lowest in the preeclamptic pregnant women (6.00±1.33) and highest in the eclamptic pregnant women (10.64±0.80), however there was a significantly difference ($p > 0.05$) in its levels among the study subjects. There is no significant difference ($p > 0.05$) in the mean values of platelets,mean platelet volume, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red cell distribution width, platelet distribution widththe study groups; but however Significant difference ($p > 0.05$) was observed in the levels of large platelet concentration ratio (LPCR) between pregnant women with preeclampsia and the other study groups.

4.5 Relationship between Calcium and Other Serum Electrolytes of Pregnant Women Normotensive, Hypertensive, proteinuric pre-eclamptic and eclamptic (N= 60)

A steady negative relationship was observed in values recorded for total calcium, ionized calcium, non ionised calcium and some electrolyte in both cases and control. However only ionised calcium and total calcium showed significant negative relationship in all the study groups, most of which was observed in the hypertensive subjects and preeclamptic subjects only. Bicarbonate show significant ($R^2= 0.694$) positive relationship with ionised calcium only in the hypertensive pregnant women (group II).

Table 4.6 Serum Protein Levels of Pregnant Women Normotensive, Hypertensive, proteinuric pre-eclamptic and eclamptic (N= 60)

Parameters	Study Groups			
	Normotensive (n =15)	**Hypertensive (n =15)**	**Preeclamptic (n =25)**	**Eclamptic (n =5)**
Total Protein(g/dl)	5.16±0.34[a]	5.84±0.53[a]	6.21±0.45[a]	6.20±0.82[a]
Albumin(g/dl)	3.94±0.28[a]	4.11±0.24[a]	3.71±0.29[a]	3.25±0.36[a]

Values are means ± SD. Values with different superscript denote significant difference along the rows ($P < 0.05$)

Table 4.7 Haematological Parameters of Pregnant women Normotensive, Hypertensive, proteinuric pre-eclamptic and eclamptic (N= 60)

Parameters	Study Groups			
	Normotensive (n =15)	**Hypertensive (n =15)**	**Preeclamptic (n =25)**	**Eclamptic (n =5)**
PCV (l/l)	33.53±0.68[a]	32.53±1.54[a]	34.36±0.6[a]	40.40±3.66[b]
WBC x 10^9/l	9.26±0.88[a]	8.33±0.87[ab]	10.64±0.80[b]	6.00±1.33[b]
PLTS	309.30±27.40[a]	286.00±23.20[a]	295.00±18.30[a]	306.00±46.50[a]
RBC x 10^{12}/l	3.99±0.12[a]	3.79±0.17[a]	4.23±0.15[a]	3.72±0.15[a]
MCV (fl)	84.62±8.54[a]	90.12±7.33[a]	88.28±8.01[a]	92.06±7.32[a]
RDW (%)	14.64±1.86[a]	14.96±1.24[a]	14.33±1.12[a]	14.84±1.24[a]
MPV (fl)	7.92±1.10[a]	7.72±1.01[a]	7.59±1.06[a]	8.40±1.29[a]
PDW (fl)	11.11±0.98[a]	11.42±1.47[a]	10.98±1.24[a]	11.86±2.11[a]
PCT (%)	0.95±3.05[a]	0.17±0.02[a]	0.17±0.36[a]	0.15±0.38[a]
LPCR (%)	15.88±5.02[a]	15.44±5.99[a]	15.81±5.8[a]	21.88±5.28[b]
HGB (g/dl)	10.92±0.76[a]	10.89±1.52[a]	10.73±1.20[a]	10.38±1.40[a]
MCH (pg)	28.95±3.76[a]	29.54±2.71[a]	29.52±2.96[a]	28.18±2.59[a]
MCHC (g/dl)	33.45±2.52[a]	32.58±1.19[a]	33.59±2.01[a]	33.64±2.30[a]
LYM x 10^9/l	2.32±0.34[a]	2.01±0.43[a]	2.19±0.42[a]	2.34±0.48[a]
GRA x 10^9/l	5.18±2.34[a]	6.04±3.55[a]	5.84±3.09[a]	4.46±2.08[a]

Values are means ± SD. Values with different superscript denote significant difference along the rows ($P < 0.05$).
Where PCV -Packed cell volume, WBC-White blood cells, RBC-Red blood cells, PLT –Platelet, MPV-Mean platelet volume, MCV-Mean corpuscular volume , MCH-Mean corpuscular hemoglobin, MCHC -Mean corpuscular hemoglobin concentration, RDW-Red cell distribution width , LPCR-Large platelet concentration ratio, PDW-Platelet distribution width, PCT-Procalcitonin, LYM-Lymphocyte, GRA- Granulocyte.

Table 4.8 Relationship of Calcium and Other Serum Electrolytes of Pregnant Women Normotensive, Hypertensive, proteinuric pre-eclamptic and eclamptic (N= 60)

Groups	Na^+				K^+				Mg^{2+}			
	I	**II**	**III**	**IV**	**I**	**II**	**III**	**IV**	**I**	**II**	**III**	**IV**
iCa	0.223	-0.938**	0.167	-0.680	0.214	-0.112	-0.656**	0.018	0.059	0.031	-0.546**	0.671
nCa	-0.334	-0.496	0.060	-0.854	0.395	0.139	-0.355	-0.116	0.420	0.014	-0.325	0.517
Tca	-0.037	-0.844**	0.151	-0.775	0.386	0.022	-0.645**	-0.050	0.420	0.014	-0.544**	0.599
Groups	Cl^-				HCO_3^-				**PTH**			
	I	**II**	**III**	**IV**	**I**	**II**	**III**	**IV**	**I**	**II**	**III**	**IV**
iCa	-0.668**	-0.938**	0.005	0.368	0.113	0.694**	-0.215	-0.249	0.184	0.031	-0.205	0.395
nCa	0.060	-0.496	-0.118	0.435	-0.148	0.217	0.018	-0.290	0.141	0.014	-0.301	0.386
Tca	-0.249	-0.839**	-0.049	0.406	-0.056	0.588	-0.148	-0.272	0.085	0.048	-0.292	0.395

Where TCa, iCa and nCa represent total, ionized and non-ionized calcium. and I, II, III and IV represent Normotensive, Hypertensive, Pre-eclampsia and Eclampsia respectively.

**Correlation is significant at the 0.05 level

CHAPTER 5

DISCUSSION

The Hausa women constitute the majority of the study; this may be due to the location of the study area and this observation is consistent with the work of Abubakar *et al.* (2009). 48% of the study groups with primary education are from the women with preeclampsia, none of the pregnant women is without a formal education, contrary to some reports that illiteracy maybe one of the predisposing factor to the development of proteinuric preeclampsia and eclampsia, Majority of the study subjects were traders and housewives and they live on monthly income of less than N5000.The age range of 26-30 years was highest (60%) in the women with eclampsia, while the percentage age of less than 20 years was low in all the study groups. Most of the subjects got married between the ages of 21-30 years, disagreeing with the report (Abubakar *et al.,* 2009) that early marriage maybe a risk factor for the development of preeclampsia and eclampsia. There is no difference between the percentage of civil servant among normotensive (40%) and hypertensive pregnant women (40%) whereas the preeclamptic pregnant women were mostly housewives (40%). This finding is in agreement with the studies of Abubakar *et al.,* (2009).

There was no significant difference ($p < 0.05$) between the mean arterial blood pressure of the pregnant women with preeclampsia (121.34±8.02) and those with eclampsia (131.34±6.07) when compared to the mean arterial pressure of the pregnant hypertensive (109.20±10.54) and pregnant normotensive (88.34±12.59) women, this could be due to the low dietary intake of calcium as low calcium intake results in production of high parathyroid hormone levels and increased membrane permeability to calcium, resulting in increased intracellular calcium levels and decreased serum calcium

levels. The former triggers vascular smooth muscle contraction and contributes to increased blood pressure seen in the preeclamptic and eclamptic pregnant women in this study (Belizan *et al.,* 1988).

Dietary intake of calcium was lower than the RDA for pregnant women in all the study group, this may be responsible for the low levels of serum calcium seen in this study. Low dietary sodium and energy intake seen in most of the study group could be as a result of dietary and caloric restriction advocated by some health workers during pregnancy, these findings is consistent with work of Rebulla, (2001). Sodium transport is altered across the cell membrane and this leads to the accumulation of sodium in the extravascular spaces and a decrease in the sodium levels. The serum sodium levels tend to decline in cases of preeclampsia as the disorder increases in severity (Searcy, 1969). The intrarenal production of cyclic GMP, endothelin and PGE2 are all decreased in preeclampsia and this may have implications in the sodium retention, hypertension, intrarenal thrombosis and the vasospasm of preeclamptic pregnancy (Clarke, 1997). Dietary vitamin E intake was higher than the recommended daily allowance for both the case and control subjects however it has been demonstrated that vitamin E supplementation did not significantly reduce the risk of any primary, secondary, or composite maternal or fetal outcome (Roberts *et al.,* 2010). Conversely the mean dietary vitamin C was higher than the RDA for pregnant women in both the case and control subjects more so other studies (Agustín *et al.,* 2011) have shown that vitamin C have some preventive properties in women with preeclampsia, though there was no consensus. Protein, fat, iron, carbohydrate and dietary fiber intake were above the RDA in all the study group however dietary intake of potassium, sodium and magnesium were lower than the RDA for pregnant women in all the study groups except for the normotensive pregnant women with dietary sodium intake of (54167.50±17183.7) and

hypertensive pregnant women with dietary magnesium intake of (421.20±371.54) which are above the recommended daily allowance levels for magnesium and sodium.

The main finding of this study was a significant reduction in the levels of serum calcium, this finding agree with earlier reports by Duly *et al.,* (2004). In the present study, a significant decrease in serum total, non ionised and ionised calcium levels was seen in the hypertensive control (2.18±0.05, 1.09±0.01 and 1.06±0.03) as compared to the normotensive controls (2.44±0.02, 1.24±0.01 and 1.18±0.01), with a further highly significant decrease in the preeclampsia (2.23±0.07, 1.32±0.03 and 0.91±0.05) and eclampsia (1.25±0.51, 0.82±0.26 and 0.43±0.25). This indicates an association between calcium deficiency and development of preeclampsia/eclampsia. This contention is amply supported by a few other studies (Mohieldein, 2007 and Punthumapol, 2008). Seely *et al* (1992) confirmed that preeclamptic women had lower serum ionized calcium levels than normotensive, third trimester, pregnant women. This reduction could be as a result of the increased calcium requirement for foetal bone formation during pregnancy. Despite the low circulating calcium levels, the intracellular level of calcium ions is high, which leads to hypertension (Belizan *et al.,* 1988). Adjustment in calcium metabolism seems to involve increasing PTH activity, which enables the pregnant woman to maintain essentially constant serum calcium levels (Reitz *et al.,* 1977).

Parathyroid hormone levels of normotensive (21.96±0.46) women in this study was significantly different ($p > 0.05$) from that of the pregnant women with preeclampsia (29.46 ±1.20) which disagree with the reports of (Mohieldein, 2007 and Punthumapol, 2008) that preeclamptic and normotensive pregnant women had equivalent levels of parathyroid hormone. The increased levels of PTH seen in this study and low ionised calcium may contribute to the increased vascular sensitivity which is documented in preeclampsia (Seely *et al.,* 1992). Also the elevation of parathyroid hormone causes an

increase in free intracellular ionized calcium, which results in vasoconstriction and a rise in the blood pressure (Idumati *et al.,* (2011). Ohara *et al* reported similar observations as the present study, where he found a significant increase of PTH in severe pregnancy induced hypertension case (Ohara *et al.,* 1986). The increased level of PTH in both groups maybe due to the following: the growing fetus which demands calcium of approximately 30 grams at term (Pitkin and Gebhardt 1977) or the increase in GFR or the increase in estrogen level, thus lowering Calcium (Howarth *et al.,* 1977).

There were elevated levels of serum magnesium in the preeclamptic pregnant women (3.22±0.69) and eclamptic pregnant women (4.66±0.80) when compared to the normotensive (1.93±0.13) and hypertensive pregnant (1.99±0.10) women; this could be as a result of the magnesium supplementation/therapy to reduce the pressure in the blood vessels. Therapeutic magnesium sulphate which is used in preeclampsia/eclampsia inhibits phosphatidyl inositol-4, 5-bisphosphate specific phospholipase C activity and subsequent calcium release in the cells, thus leading to decreased intracellular calcium levels and a decrease in blood pressure (Indumati *et al.,* 2011).

Idumati *et al.,* (2011) observed that the serum Na^+ concentration was lower in the preeclamptics (135.9 ± 1.07) than in the normotensive pregnant women (136.40 ± 0.66), this findings is in disagreement with the present study however it was observed by Kashyap *et al.,* (2006) that the concentration level of serum Na^+ or HCO_3^- had definitely higher values in the preeclamptics than for the normotensive group which is in agreement with this study, however serum chloride levels was significantly lower in the normotensive pregnant women (98.50 ± 0.58) compared to the women with preeclampsia (102.40 ± 0.55).

Decrease in serum albumin was observed in preelamptic (3.71±0.29) and eclamptic (3.25±0.36) pregnant women when compared to the normotensive (3.94±0.28) and hypertensive (4.11±0.24) pregnant women, this decrease in serum albumin maybe due to hemodilution; the albumin remains low until parturition. Less well appreciated is that the fall in albumin causes the total serum calcium to fall to levels normally associated with symptomatic hypocalcemia (Haller *et al.,* 1989). The total calcium includes albumin-bound, bicarbonate-and-citrate-complexed, and ionized or free fractions of calcium.

The white blood cells of the pregnant normotensive and hypertensive pregnant women was significantly ($P < 0.05$) higher than that of the proteinuric preeclampsia and eclampsia subjectin this study when compared to the earlier report by Alisi *et al*. (2011) this could be due to their immune compromised state, whereas the concentrations of packed cell volume, platelet and Red blood cell count agree with the observations of Canoneri *et al.,* (2009). Though little or nothing has been reported on other haematological parameters of women with preeclampsia and eclampsia, there was no significant difference ($P > 0.05$) in their levels between the case and control subjects except for the large platelet concentration ratio which showed significant difference ($P < 0.05$) between the case and control subjects.

The correlation of some of the electrolytes and serum calcium levels show that Hypertensive and Normotensive pregnant women have significant negative relationship between serum ionize calcium , serum total calcium and serum sodium, potassium, magnesium and chloride, while only the hypertensive pregnant women show strong positive relationship between bicarbonate and ionized calcium

CHAPTER 6

SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 Summary

i. Majority of the pregnant women that participated in the study were Hausas and are within the marrriage age range of 26-30 years, many were housewives irrespective of their educational status and they survive on monthly income of <₦5000. Mean arterial blood pressure was significantly different in all the study groups

ii. The mean energy, water, potassium, vitamin E and calcium intake was below the recommended dietary allowance, this decreased in calcium intake may leads to an increase in the parathyroid hormone, which increases intracellular calcium, thus leading to an increase in the vascular smooth muscle contraction and thus, an elevation in the blood pressure.

iii. There was no significant change in the sodium, chloride and potassium levels in the preeclampsia/eclampsia cases as compared to those in the normal pregnancy cases ($P<0.05$). However, there was a linear fall in serum total, ionised and non-ionised calcium levels all the studygroups. This decrease in total serum calcium maybe due to the physiologic hypoalbuminemia secondary to the normal expansion of the intravascular volume observed early in pregnancy. This is consistent with the work by Pitkin, (1985) and Seki *et al.,* (1991). Ionized calcium levels, however, remain unchanged throughout gestation. The PTH remain low though normal during the third trimester and increase towards parturition.

iv. There was no significant difference in the levels of serum protein in all the study groups

v. A steady positive relationship was observed in the levels of calcium and some other electrolytes, though few were significant.

6.2 Conclusion

It has been widely documented that there is a relationship between low calcium level and preeclampsia. Our study also suggests that maternal serum calcium and PTH are related to preeclampsia and eclampsia. The low level of maternal serum calcium may have a role in the development of this disorder in pregnancy and may be used as predictor risk for pre-eclampsia.

Although literatures indicate early marriage as a contributing factor to the development of preeclampsia and eclampsia in the pregnant women, age of marriage of subjects in this study range from 26-30 years and therefore early marriage may not be a contributing factor to the development of preeclampsia and eclampsia.

No significant difference was observed in the Na, K and Cl levels; however there was a significant difference in the levels of serum total, ionized and non-ionised calcium relative to the levels of serum parathyroid hormone. There was no significant change in the levels of Haematological parameters among the study groups except in the white blood Cell count and large platelet concentration ratio. There was no significant difference in the levels of serum protein among the study group. Dietary calcium, vitamin E and energy are lower than the RDA for pregnant women in all the study groups. Only the calcium and some haematological parameters show significant correlation.

6.3 Recommendation

i. Further study on genetic implication on the etiology of preeclampsia/eclampsia with larger sample size and frame should be conducted.

ii. Possible difference in the vitamin D levels, minerals and lipid metabolism of women with hypertensive disorder in pregnancy can be explored in future studies.

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APPENDIX I

CALCIUM METABOLIC INDICES OF WOMEN WITH PRECLAMPSIA AND ECLAMPSIA

STUDY QUESTIONNAIRE

SECTION 1: Patient information

1. Ethnicity 1=Hausa 2= yoruba 3= Igbo 4= others
2. Weight **[ ]**
3. Height [ ]
4. BMI [ ]
5. Bp[ ]mmHg
6. Age [ ]
7. Age at marriage [ ]
8. Do have any History of hypertension in pregnancy: 1= Yes 2=No
9. If yes, how many times 1= one 2= two 3= three 4= four 5= ≥ five
10. What is your main occupation?

    1=Civil servant 2=Trader 3= Farming 4=Full time house wife 5= Others (specify)____
11. Have you given birth before? 1= yes 2= No
12. If yes how many times? 1= 1-3 2= 4-7 3= 7-10 4= > 10
13. Highest educational Status attained

    1=Islamiyya 2= Primary 3= Secondary 4= Tertiary 5= None
14. Do you have family history of any of the following diseased conditions?

    1=Hypertension 2= Diabetes 3= Kidney disease 4= None
15. If yes who among your family members

    1=Father 2= Mother 3=Grandmother 4= Others (specify)_____
16. Can you remember if any of your close relatives ever developed pre-eclampsia/eclampsia during the course of her pregnancy?

    1=Yes 2= No
17. If yes, who?

    1=Mother 2=Aunties 3=Maternal Cousins

    4=Paternal cousins 5=others (specify)_____

18. Do you freely Move around ?

    1=Yes 2=No

19. Do you have income generating activities at home? 1= yes 2= No

20. If yes, what kind of income generating activities do you do for yourself?

    1=Petty trading

    2=Islamiyya teacher

    3=Weaving

    4= others (specify) _____

21. Do you totally depend on your husband for treatment of minor ailment?

    1=Yes 2=No

22. Have you been attending antenatal clinics during the course of your pregnancy? 1=yes 2= No

23. How many wives does your husband have? 1= one 2= two 3= three 4= four 5= ≥ five

24. Which position do you occupy? 1= one 2= two 3= three 4= four 5= others

25. Estimated Monthly Income

    1=< N5,000 2=N5,000-N14,999 3=N15,000-24,999 4=N25,000-N34,999

    5=N35,000-N44,999 6=N45,000-N54,999 7= > N55,00

APPENDIX II

24-Hour Dietary Recall Protocols

Materials used

Three pass recall method was used as described (Bialostosky, 2002)

1. 24-hour recall form and a pencil
2. Food Recall Kit containing each of the following:

- ✓ Measuring cups and spoons (Liquid and Dry)
- ✓ Food Models
- ✓ Different sizes of Plates use as a visual to measure out portions or determine how large a portion of food consumed might be.
- ✓ Picture of different kinds and sizes of Nigerian foods

TIME	FOODS	QUANTITY	DESCRIPTION

APPENDIX III

List of Components of Elisa Kit

Component	Amount
Microwells coated with Sterptavidin	12x8x1
Biotinylated PTH Antibody (reagent 1)	7.0 ml
Peroxidase (Enzyme) labeled PTH Antibody (reagent 2)	7.0 ml
TMB Substrate (reagent B)	20 ml
Diluent [equine serum] for Patient Samples read off -scale (reagent 3)	2 ml
ELISA Wash Concentrate [Saline with surfactant] (reagent A)	30 ml
Stop Solution (SOLN)	20 ml
Reconstitution Solution containing surfactant (reagent 4)	5 ml
Calibrators (A,B,C,D,E,F) (CAL)	0.5 ml
Controls 1 & 2 (CTRL)	0.5 ml

APPENDIX V

Correlation Graphs of Calcium and Some Other Serum Electrolytes of Pregnant Women with Preeclampsia and Eclampsia

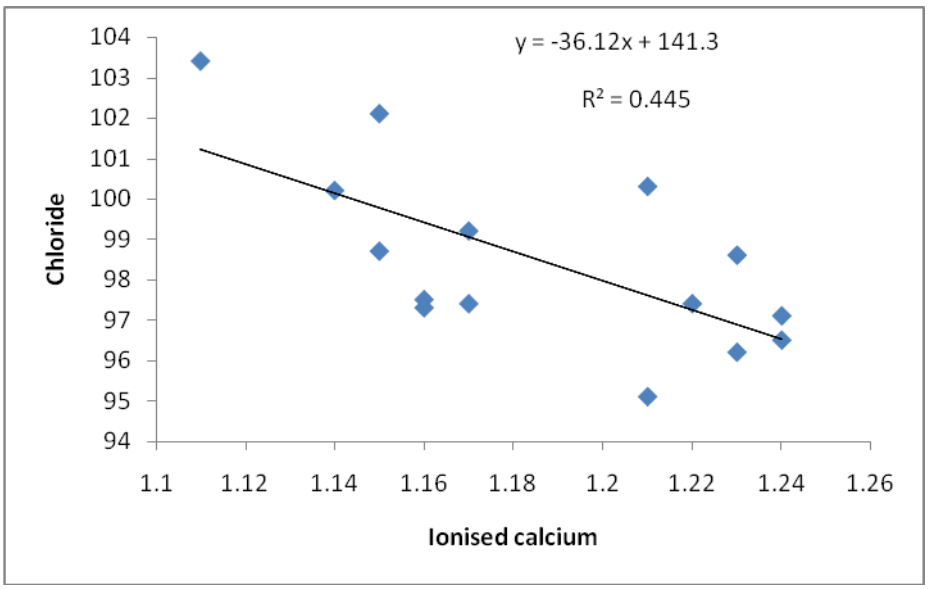


Figure 4.2 Correlation of ionized calcium with Chloride for Normotensive pregnant women

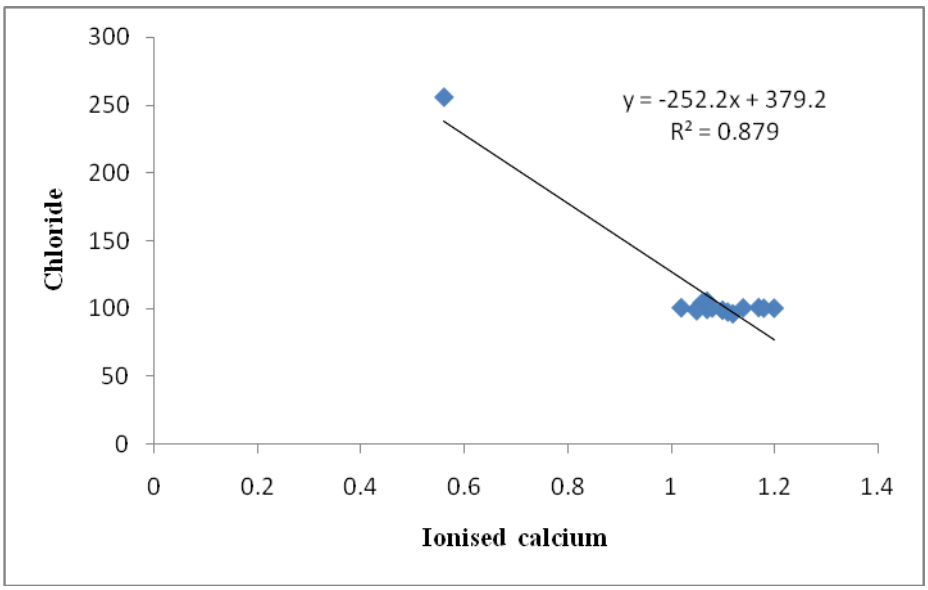


Figure 4.3 Correlation of ionized calcium with Chloride for hypertensive pregnant women

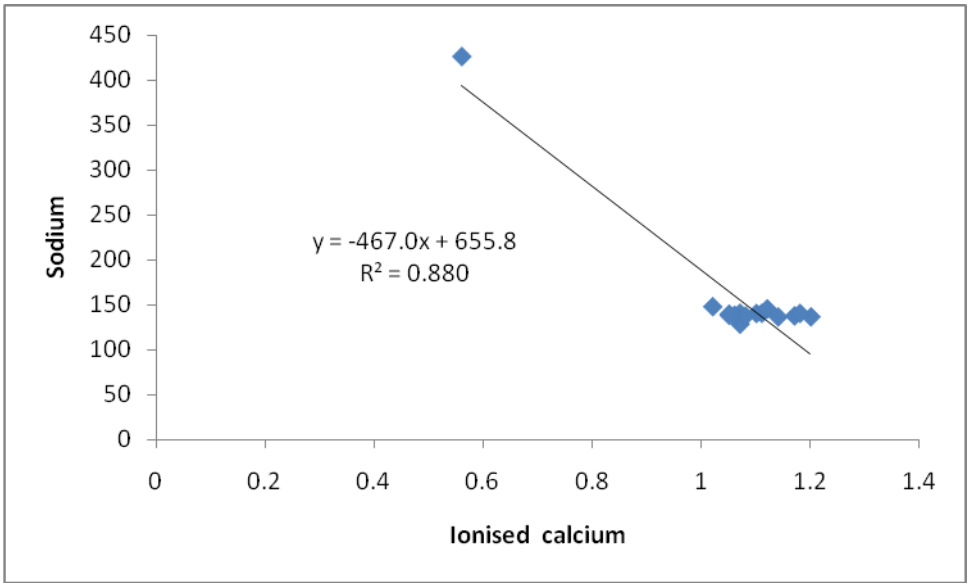


Figure 4.4 Correlation of ionized calcium with Sodium for hypertensive pregnant women

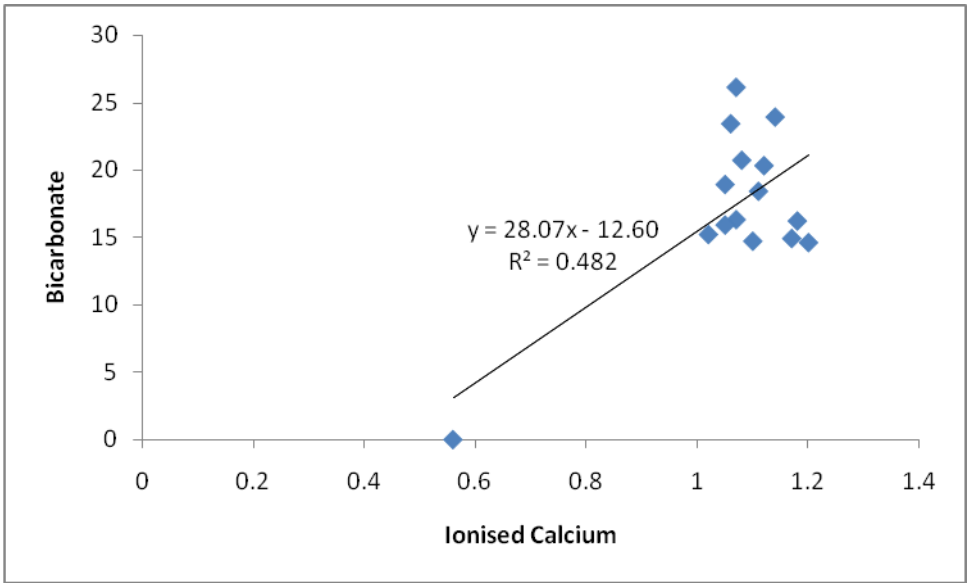


Figure 4.5 Correlation of ionized calcium with bicarbonate for hypertensive pregnant women

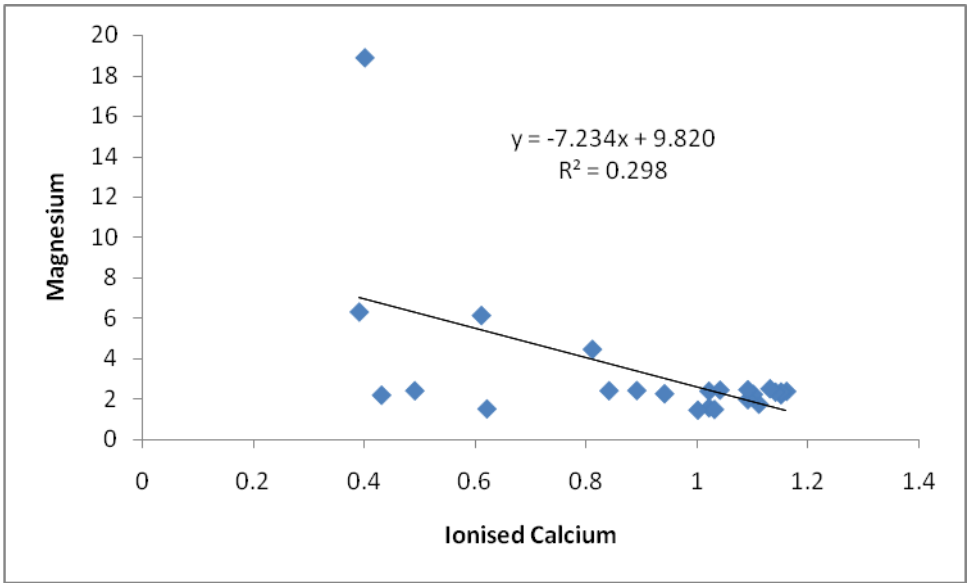


Figure 4.6 Correlation of ionized calcium with Magnesium for preeclamptic pregnant women

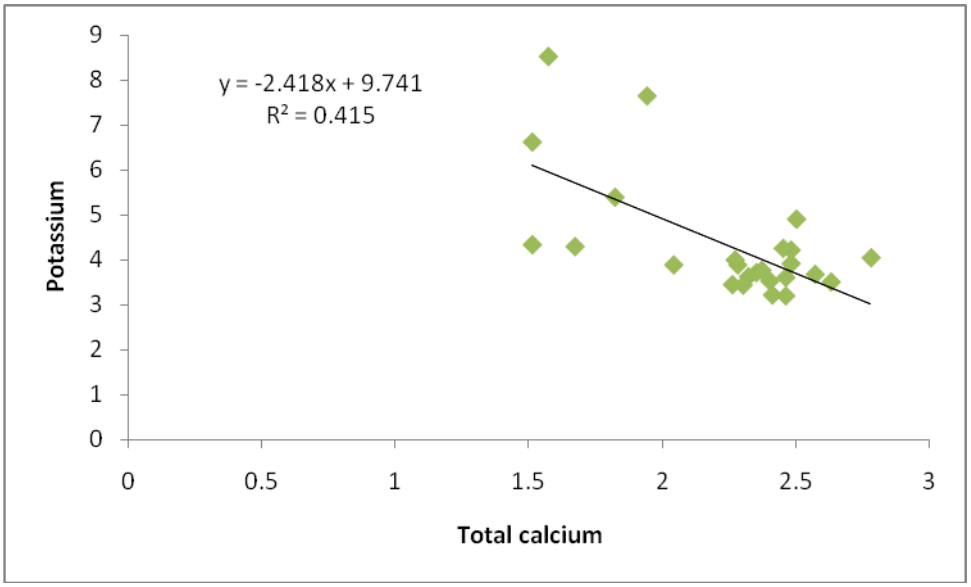


Figure 4.7 Correlation of total calcium with potassium for hypertensive pregnant women

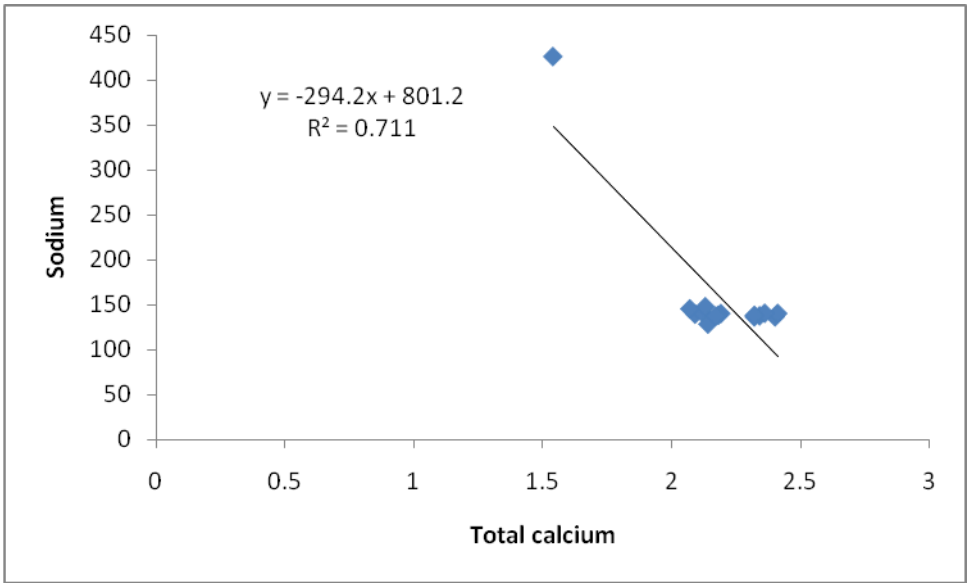


Figure 4.8 Correlation of total calcium with sodium for hypertensive pregnant women

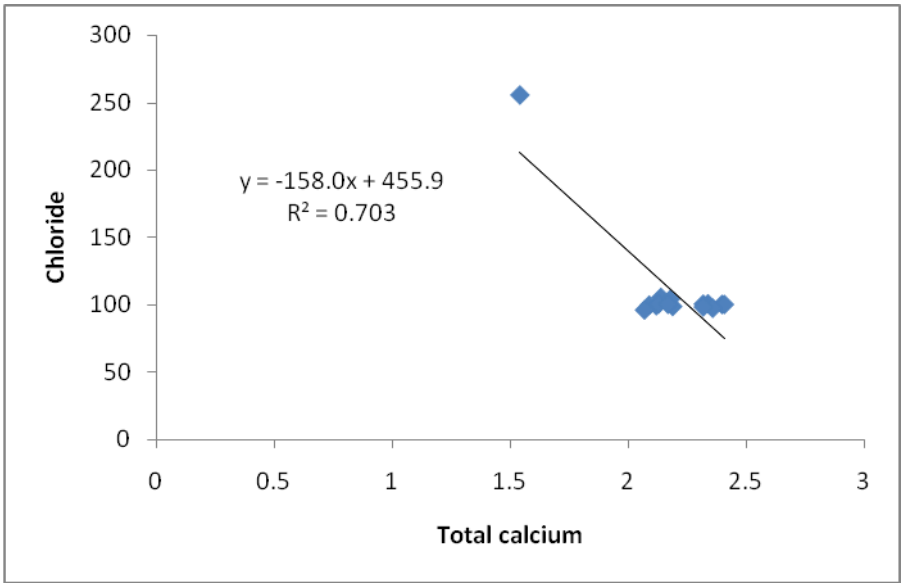


Figure 4.9 Correlation of total calcium with chloride for hypertensive pregnant women

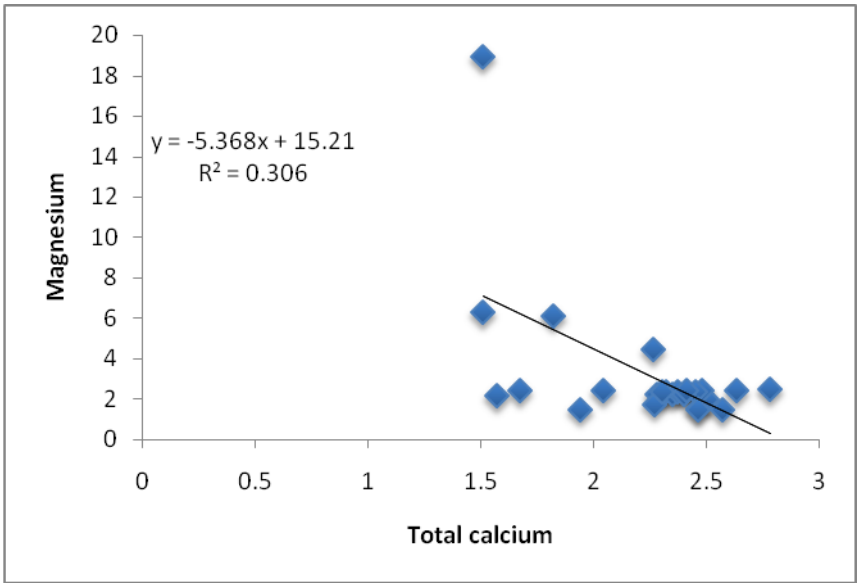


Figure 4.10 Correlation of total calcium with magnesium for preeclamptic pregnant women

APPENDIX VI

Ahmadu Bello University Teaching Hospital

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Director of Administration: **BARR. ISHAK BELLO,** *LL.B, BL, LL.M, PGDM, AHAN, FCAI*

Our Ref: ABUTH/HREC/TRG/36

7th March, 2013

Shehu Muhammad Hassan
Dept of Biochemistry
ABU Zaria

ETHICAL CLEARANCE.

Your application for ethical clearance on the research proposal titled: - **'Calcium metabolic indices of women with preeclampsia and eclampsia''** refers.

This is to convey ethical approval for you to commence the study. The ABUTH Scientific and Health Research Committee require an annual update from the Principal Investigator.

Thank you.

PROF. J.U OKPAPI
CHAIRMAN, ABUTH HREC