

ELECTROLYTE PHYSIOLOGY OF SELECTED SERUM ELECTROLYTES AND
NITRIC OXIDE IN PREECLAMPTICS AT THE KORLE-BU TEACHING HOSPITAL

BY

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DECLARATION

I Ebenezer Owusu Darkwa, author of this thesis do hereby declare that, with the exception of references to other people's work which has been duly cited, this work has entirely resulted from my personal original research under the supervision of Rev. Dr. Charles Antwi-Boasiako and Prof. Samuel A. Obed and has not been presented for another degree elsewhere.

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DEDICATION

I dedicate this thesis to Jesus Christ whose I am and whom I serve and to all the patients who participated in the study.



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May the almighty God bless you all.

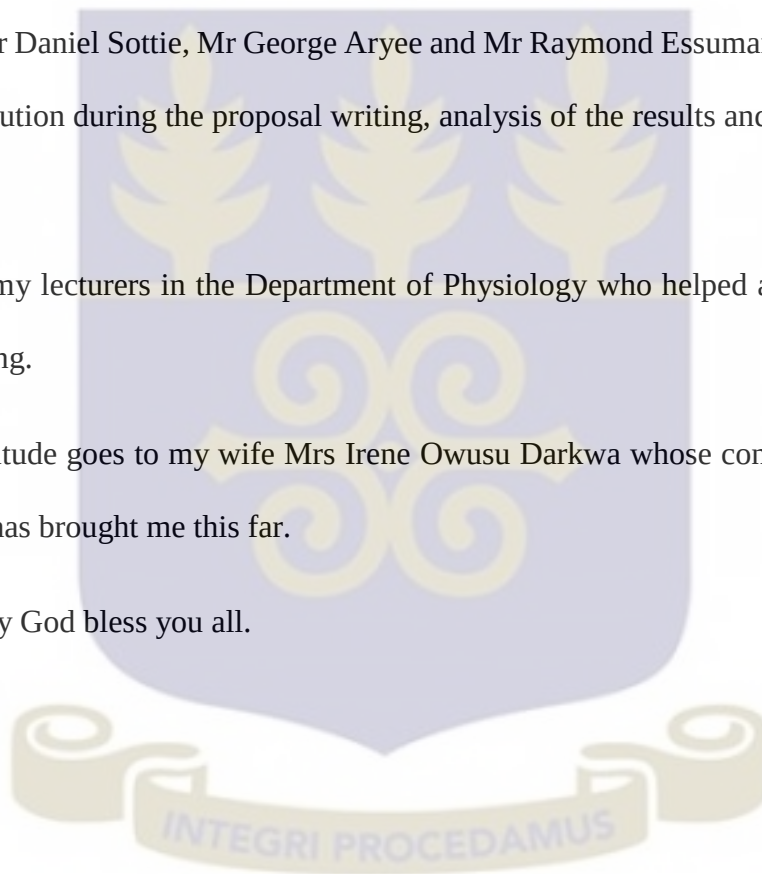


TABLE OF CONTENTS

DECLARATION	i
DEDICATION	ii
ACKNOWLEDGEMENT	iii
TABLE OF CONTENTS	iv
LIST OF FIGURES	x
LIST OF TABLES	xii
LIST OF ABBREVIATIONS	xiii
ABSTRACT	xvi
CHAPTER ONE	1
1.0 INTRODUCTION	1
1.1 Background of Study	1
1.2 Problem Statement	6
1.3 Justification/Relevance	7
1.4 General Aim	8
1.5 Objectives	8
CHAPTER TWO	9
2.0 LITERATURE REVIEW	9
2.1 Pre-eclampsia	9
2.2 Classification of Hypertensive Disorders of Pregnancy	10
2.2.1 Chronic Hypertension	10
2.2.2 Gestational Hypertension	10
2.2.3 Preeclampsia	11

2.2.4 White-coat Hypertension	12
2.3 Epidemiology	13
2.4 The Fetoplacental Unit	14
2.5 Pathophysiology of Pre-eclampsia	15
2.5.1 Normal Placentation	16
2.5.2 Abnormal Placentation	17
2.6 Risk factors of Pre-eclampsia.....	20
2.7 Clinical Features and Diagnosis of Preeclampsia	22
2.8 The role of major cations in Pre-eclampsia.....	22
2.8.1 The role of Calcium in Pre-eclampsia	22
2.8.2 The role of Magnesium in Pre-eclampsia.....	24
2.8.3 The role of Serum sodium and potassium in Pre-eclampsia	26
2.9 The role of nitric oxide in Pre-eclampsia	27
2.10 Prevention of Preeclampsia.....	30
CHAPTER THREE	32
3.0 METHODOLOGY	32
3.1 Study Design	32
3.2 Study Site	32
3.3 Target Population	33
3.4 Case Definition.....	33
3.5 Inclusion Criteria.....	33
3.6 Exclusion Criteria.....	34

3.7 Sampling Technique.....	35
3.8 Sample Size Determination.....	36
3.9 Procedure.....	36
3.9.1 Participants demographics	36
3.9.2 Measurement of blood pressure.....	36
3.9.3 Collection of blood samples, preparation and storage.....	37
3.9.4 Measurement of haematological profile	37
3.9.5 Procedures for estimation of serum magnesium, calcium, potassium and sodium levels.....	38
3.9.6 Measurement of nitric oxide.....	39
3.9.7 Preparation of a Nitrite Standard Reference Curve	39
3.9.8 Determination of Nitrite concentration as an index for nitric oxide in experimental samples	40
3.10 Data Handling and Statistical analysis	41
3.11 Dissemination of results.....	41
3.12 Ethical consideration.....	42
3.13 Consent.....	42
CHAPTER FOUR.....	43
4.0 RESULTS	43
4.1 General/Basic Sample Characteristics	43
4.2 Comparing mean serum electrolyte and nitric oxide levels of preeclamptic, normotensive pregnant women and normotensive non-pregnant women	46

4.3 Comparing mean serum electrolyte and nitric oxide levels of first, second and third trimester normotensive pregnant women	48
4.4 Comparing mean arterial pressure of preeclamptic, normotensive pregnant women and normotensive non-pregnant women	50
4.5 Comparing mean heart rate of preeclamptic, normotensive pregnant women and normotensive non-pregnant women	51
4.6 Comparing mean arterial pressure of first, second and third trimester normotensive pregnant women	52
4.7 Comparing mean heart rate of first, second and third trimester normotensive pregnant women	53
4.8 Comparing change in mean heart rate and mean arterial pressure of first, second and third trimester normotensive pregnant women	54
4.9 Comparing mean serum magnesium levels in preeclamptic, normotensive pregnant women and normotensive non-pregnant women	55
4.10 Comparing mean serum magnesium levels in first, second and third trimester normotensive pregnant women	56
4.11 Comparing mean serum total calcium levels in preeclamptic, normotensive pregnant women and normotensive non-pregnant women	57
4.12 Comparing mean serum total calcium levels for first, second and third trimester normotensive pregnant women	58
4.13 Comparing mean serum ionised calcium levels for preeclamptic, normotensive pregnant women and normotensive non-pregnant women	59

4.14 Comparing mean serum ionised calcium levels for first, second and third trimester normotensive pregnant women	60
4.15 Comparing mean serum sodium levels for preeclamptic, normotensive pregnant women and normotensive non-pregnant women	61
4.16 Comparing mean serum sodium levels for first, second and third trimester normotensive pregnant women	62
4.17 Comparing mean serum potassium levels for preeclamptic, normotensive pregnant women and normotensive non-pregnant women	63
4.18 Comparing mean serum potassium levels for first, second and third trimester normotensive pregnant women	64
4.19 Comparing mean serum nitric oxide levels for preeclamptic, normotensive pregnant women and normotensive non-pregnant women	65
4.20 Comparing mean serum nitric oxide levels for first, second and third trimester normotensive pregnant women	66
CHAPTER FIVE	67
5.0 DISCUSSION	67
5.1 Introduction	67
5.2 Changes in mean arterial pressure (MAP) and heart rate (HR) during normal pregnancy and preeclampsia	68
5.3 Serum Magnesium Levels and Preeclampsia	69
5.4 Serum Calcium Levels and Preeclampsia	72
5.5 Serum Sodium Levels and Preeclampsia	76
5.6 Serum Potassium Levels and Preeclampsia	79

5.7 Serum Nitric Oxide Levels and Preeclampsia.....	81
CONCLUSIONS.....	85
RECOMMENDATIONS.....	86
LIMITATIONS OF THE STUDY.....	86
REFERENCES	87
APPENDIX.....	122
Appendix 1: Informed Consent Form	122
Appendix 2: Data Collection Sheet.....	124
Appendix 3: Comparison of the means of demographic, clinical and basic laboratory data for preeclamptic, normotensive pregnant and normotensive non-pregnant women (Table 4.1)	125
Appendix 4: Comparison of the means of serum electrolytes and nitric oxide of preeclamptic, normotensive pregnant and normotensive non-pregnant women (Table 4.2).....	128
Appendix 5: Comparison of the means of serum electrolytes of first, second and trimester normotensive pregnant women (Table 4.3).....	129



LIST OF FIGURES

Figure	Page
Figure 2.1: A figure showing normal (A) placentation in normal pregnancy compared to abnormal (B) placentation in preeclampsia.	15
Figure 4.1: Mean arterial pressure (MAP) of preeclamptic, normotensive pregnant and normotensive non-pregnant women	50
Figure 4.2: Mean heart rate in preeclamptic, normotensive pregnant and normotensive non-pregnant women	51
Figure 4.3: Mean arterial pressure (MAP) of first, second and third trimester normotensive pregnant women	52
Figure 4.4: Mean heart rate (HR) of first, second and third trimester normotensive pregnant women	53
Figure 4.5: A line graph comparing change in mean heart rate (HR) and mean arterial pressure (MAP) of first, second and third trimester normotensive pregnant women	54
Figure 4.6: Mean serum magnesium levels in preeclamptic, normotensive pregnant and normotensive non-pregnant women	55
Figure 4.7: Mean serum magnesium levels in first, second and third trimester normotensive pregnant women	56
Figure 4.8: Mean serum total calcium levels in preeclamptic, normotensive pregnant and normotensive non-pregnant women	57
Figure 4.9: Mean serum total calcium levels for first, second and third trimester normotensive pregnant women	58
Figure 4.10: Mean serum ionised calcium levels for preeclamptic, normotensive pregnant and normotensive non-pregnant women	59

Figure 4.11: Mean serum ionised calcium levels for first, second and third trimester normotensive pregnant women 60

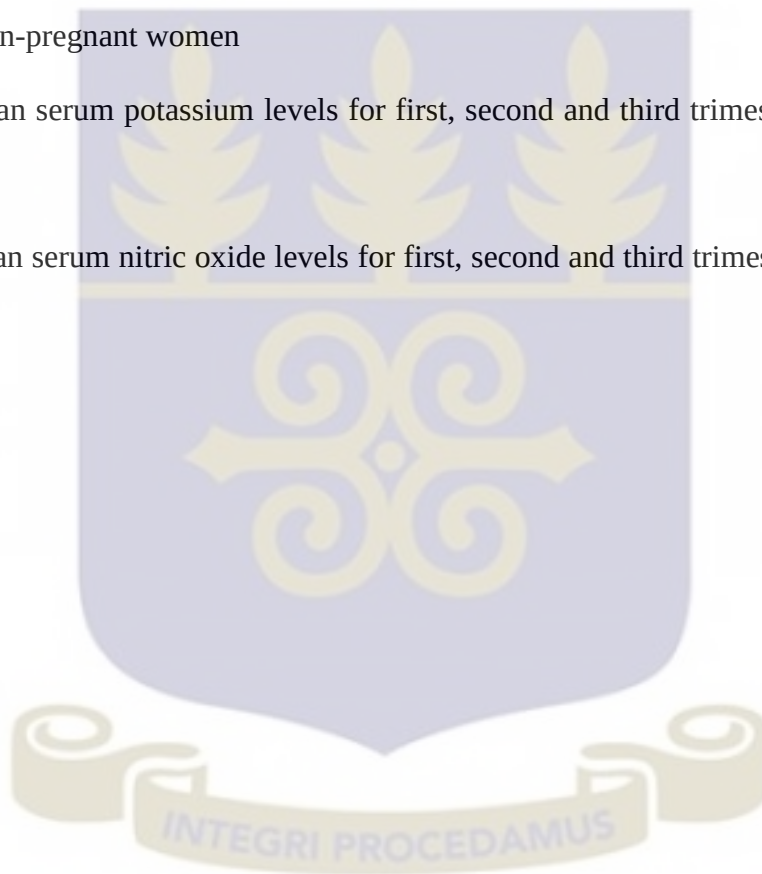
Figure 4.12: Mean serum sodium levels for preeclamptic, normotensive pregnant and normotensive non-pregnant women 61

Figure 4.13: Mean serum sodium levels for first, second and third trimester normotensive pregnant women 62

Figure 4.14: Mean serum potassium levels for preeclamptic, normotensive pregnant and normotensive non-pregnant women 63

Figure 4.15: Mean serum potassium levels for first, second and third trimester normotensive pregnant women 64

Figure 4.17: Mean serum nitric oxide levels for first, second and third trimester normotensive pregnant women 66



LIST OF TABLES

Table 4.1: Demographic, clinical and basic laboratory data for preeclamptic, normotensive pregnant and normotensive non-pregnant women	44
Table 4.2: Measured mean serum electrolytes and nitric oxide of preeclamptic, normotensive pregnant and normotensive non-pregnant women	46
Table 4.3: Measured mean serum electrolytes and nitric oxide of first, second and third trimester normotensive pregnant women	48

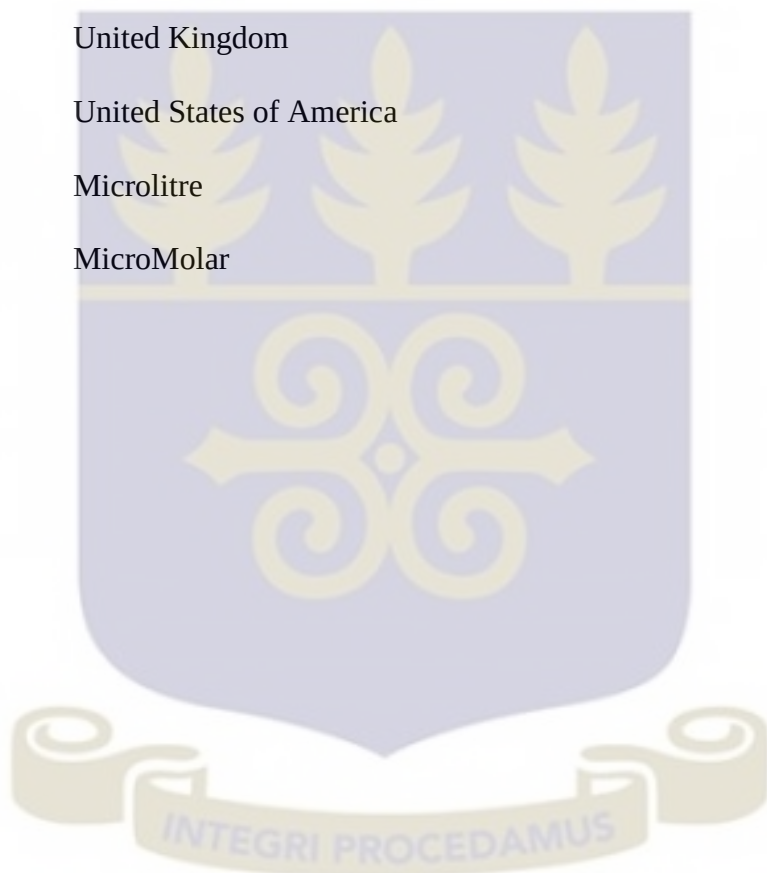


LIST OF ABBREVIATIONS

°C	Degrees Celsius
ACOG	American College of Obstetricians and Gynecologists
ADH	Antidiuretic Hormone
ADMA	Asymmetric Dimethyl Arginine
ALT	Alanine Transaminase
ANOVA	Analysis of Variance
AST	Aspartate Transaminase
ATPase	Adenosinetriphosphatase
AVP	Arginine Vasopressin
BMI	Body Mass Index
BNP	Brain Natriuretic Peptide
Ca	Calcium
Ca ²⁺ -ATPase	Calcium adenosine triphosphatase
cGMP	Cyclic Guanosine Monophosphate
CMACE	Centre for Maternal and Child Enquiries
DIC	Disseminated Intravascular Coagulopathy
DNA	Deoxyribonucleic acid
DRI	Dietary Reference Intake
g	Gram
GFR	Glomerular Filtration Rate
GmbH	Gesellschaft mit beschränkter Haftung ('company with limited liability')
GOT	Glutamate Oxaloacetate Transminase
GPT	Glutamate Pyruvate Transaminase
H ⁺ -K ⁺ α ₂ -ATPase	Hydrogen Potassium Alpha-2 Adenosinetriphosphatase

Hb	Haemoglobin concentration
HELLP	Haemolysis, Elevated Liver enzymes, Low Platelet
HNO ₃	Nitric Acid (Trioxonitrate (V) Acid)
HR	Heart Rate
IOM	Institute of Medicine
IP ₃	Inositol Triphosphate
ISSHP	International Society for the Study of Hypertension in Pregnancy
K	Potassium
M	Molar (moles per Litre)
MAP	Mean Arterial Pressure
Mg	Magnesium
mg	Milligram
ml	Millilitre
mmHg	Millimetres of Mercury
mmol/L	Millimole per Litre
Na EDTA	Sodium Ethylenediaminetetraacetate
Na	Sodium
Na ⁺ -K ⁺ -ATPase	Sodium Potassium Adenosinetriphosphatase
NED	N-1-naphthylethylenediamine
NO	Nitric Oxide
PGE ₂	Prostaglandin E ₂
PGI ₂	Prostaglandin I ₂ (prostacyclin)
PLT	Platelet Count
PTH	Parathyroid Hormone
PTHrP	Parathyroid Hormone Receptor Protein

ROS	Reactive Oxygen Species
rpm	Revolutions per minute
SD	Standard Deviation
SEM	Standard Error of Mean
sENG	soluble endoglin
SPSS	Statistical Package for the Social Sciences
STMP	Syncytiotrophoblast Microparticles
UK	United Kingdom
USA	United States of America
μl	Microlitre
μM	MicroMolar



ABSTRACT

Brief Background: Preeclampsia is a multisystem disorder in pregnancy affecting 5-14% of all pregnancies worldwide. Preeclampsia is associated with an increased risk of cardiovascular diseases later in life of both the mother and the baby. It is common but its aetiology remains unknown. It is the most common, yet least understood disorder of pregnancy. Despite a steady reduction in maternal mortality from this disorder in more developed countries, the aetiology still remains unclear, recent studies indicate that serum electrolytes and Nitric oxide (NO) levels may play a role in pre-eclampsia since it is vascular endothelial disorder. Magnesium and nitric oxide play important role in peripheral vasodilatation whiles calcium also have an indirect effect on smooth muscle function by increasing magnesium levels. However, the levels of these electrolytes and NO in normal pregnancy and preeclampsia patients are still uncertain. The aim of this study is to measure serum levels of calcium and magnesium in preeclamptic pregnancy and to compare with those in normal pregnancy.

Aim: To determine the electrolyte physiology of selected serum electrolytes and nitric oxide in preeclamptics at the Korle-Bu Teaching Hospital.

Brief Methodology: This was a case-control cross sectional study involving 162 women consisting of 30 preeclamptics, 100 normotensive pregnant women and 32 normotensive non-pregnant women conducted at the Obstetrics and Gynaecology clinic of Korle-Bu Teaching Hospital. Women in each of these categories were sampled consecutively and recruited into the study after obtaining an informed consent. Preeclampsia was diagnosed using the International Society for the Study of Hypertension in Pregnancy criteria. Blood pressure and heart rate of the participants were measured after which 5 ml of venous blood was obtained from each participant. Blood pressure was measured using a mercury sphygmomanometer and a stethoscope. Heart rate was determined by auscultation with a stethoscope and a stopwatch.

Determination of serum magnesium and calcium was done using a Flame Atomic Absorption Spectrometer (Variant 240FS manufactured by VARIAN Australia Pty Ltd). Serum sodium and potassium were analysed using Sherwood Flame Photometer (Model 420 by Sherwood Scientific Ltd). Nitric oxide was measured using Griess reagent system by ELISA method (Promega, Madison, USA).

Results: The study showed a statistically significant difference in the serum levels of magnesium, sodium and potassium in normotensive non-pregnant women (0.80 ± 0.10 , 139.16 ± 4.64 , 3.61 ± 0.51), normotensive pregnant (0.77 ± 0.14 , 139.00 ± 6.54 , 3.75 ± 0.47) and preeclamptic women (0.70 ± 0.15 , 136.13 ± 4.17 , 3.45 ± 0.54) mmol/L ($p=0.006$, $p=0.009$, $p=0.009$) respectively. There was no statistically significant difference in the serum levels of calcium (total and ionised) and nitric oxide in normotensive non-pregnant (2.20 ± 0.16 , 1.19 ± 0.09 , 470.5), normotensive pregnant (2.14 ± 0.34 , 1.20 ± 0.11 , 1308.3) and preeclamptic (2.13 ± 0.30 , 1.15 ± 0.06 , 1178.78) women ($p=0.564$, $p=0.091$, $p=0.447$) respectively.

Conclusion: This study revealed abnormalities of some selected serum electrolytes which may have a role in the pathophysiology of preeclampsia. This is evidenced by the finding of a significantly reduced serum magnesium, sodium and potassium levels in preeclampsia compared to normotensive pregnant women. However, no change was found in the serum levels of calcium (total and ionised) and nitric oxide levels in preeclampsia compared to normotensive pregnant women.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of Study

The International Society for the Study of Hypertension in Pregnancy (ISSHP) (Tranquilli *et al.*, 2014) defines preeclampsia as the onset of hypertension after 20 weeks of gestation with the coexistence of one or more of the following new-onset conditions:

- i. Proteinuria (spot urine protein/creatinine >30 mg/mmol [0.3 mg/mg] or >300 mg/day or at least 1 g/L [2+] on dipstick testing)
- ii. Other maternal organ dysfunction:
 - renal insufficiency (creatinine >90 μ mol/L; 1.02 mg/dL)
 - liver involvement (elevated transaminases – at least twice upper limit of normal $\pm$ right upper quadrant or epigastric abdominal pain)
 - neurological complications (examples include eclampsia, altered mental status, blindness, stroke, or more commonly hyperreflexia when accompanied by clonus, severe headaches when accompanied by hyperreflexia, persistent visual scotomata)
 - haematological complications (thrombocytopenia –platelet count below 150,000/dL, disseminated intravascular coagulopathy (DIC), haemolysis)
- iii. Uteroplacental dysfunction
 - foetal growth restriction

Preeclampsia is one of the commonest aetiologies of foetal and maternal mortality and morbidity (Sukonpan & Phupong, 2005). It is a multi-system disorder affecting approximately 5 – 7 % of all pregnancies worldwide and it is the commonest, yet least understood disease of

pregnancy (Ziaei, Ranjkesh, & Faghihzadeh, 2008). The incidence of preeclampsia in developing nations is estimated to be 4-18% (Villar, Betran, & Gulmezoglu, 2001). Sixteen percent (16%) of all maternal mortality in developed countries and 9% of maternal mortalities in Asia and Africa is said to be due to hypertensive disorders in pregnancy (Khan, Wojdyla, Say, Gülmezoglu, & Van Look, 2006). This difference may be due to the fact that developed countries with better health systems have reduced maternal deaths resulting from obstetric haemorrhage and infections whereas these causes continue to play a major role in developing countries (Der *et al.*, 2013; Nair *et al.*, 2015). Also, maternal deaths in developing countries are underreported, especially in rural communities. Eclampsia, HELLP syndrome (Haemolysis, Elevated Liver enzymes, and Low Platelets), hepatic failure, cerebral haemorrhage, renal failure, among others are usually the cause of the maternal deaths in preeclampsia. Eclampsia, which is a deadly complication of preeclampsia is characterised by grand mal seizures (Obed & Aniteye, 2007). Eighteen percent (18%) of 724 total maternal deaths at Korle-Bu Teaching Hospital between 1984 and 1994 were due to hypertensive disorders in pregnancy including preeclampsia (Lassey & Wilson, 1998).

Perinatal and neonatal mortality rate of 10% worldwide is associated with preeclamptic disorders with prematurity as the commonest cause of the neonatal deaths (Maynard & Karumanchi, 2011). Other complications include intrauterine foetal death and abruptio placentae, low birth weight, and children with cardiovascular abnormalities.

Whilst earlier studies suggested that preeclamptic women after delivery had no increased risk of adverse long term outcomes than non-preeclamptic women from the general population, current studies, however, suggest the reverse (Chesley, Annitto, & Cosgrove, 1976; Davison *et al.*, 2004).

Pathophysiology of preeclampsia involves both maternal and foetal factors. Although hypertension and proteinuria are the diagnostic criteria for preeclampsia, they are only symptoms of the pathophysiologic changes that occur in preeclampsia. Though aetiology of preeclampsia remains unclear, many theories suggest abnormal placental implantation and abnormal trophoblastic invasion as a possible cause (Smith & Kenny, 2006). Placental blood vessel abnormalities in early pregnancy causes a reduced blood supply to the placenta leading to hypoxia and ischaemia. Subsequently, there is a release of antiangiogenic factors into the circulation of the pregnant mother. These factors cause endothelial dysfunction in the pregnant mother leading to hypertension and other clinical features of preeclampsia. The molecular basis of this is unresolved in literature as most of these antiangiogenic factors causing placental dysregulation are under investigation. (Roberts & Cooper, 2001). Perfusion is diminished due to vascular haemoconcentration and third spacing of intravascular fluids. Preeclampsia is also accompanied by exaggerated inflammatory response and inadequate endothelial activation. Coagulation cascade activation and resultant micro thrombi formation further compromise blood flow to organs (Sibai, 2003). It has been postulated that fluctuations in maternal serum ions may be the precipitating cause of elevated blood pressures in preeclampsia (Bussen, Sütterlin, & Steck, 1999; Hanisch, Pfeiffer, Schlebusch, & Schmolling, 2004).

Dietary deficiency of mineral ions have been implicated to have a harmful effect on the pregnant mother and growing foetus and possibly complicate preeclampsia (Raman & Shatrugna, 2002). A great effect of this consequence is evident in developing countries where the diet of most pregnant women are deficient in most essential minerals (Takaya, Yamato, & Kaneko, 2006). For instance, dietary deficiency of magnesium has been established to play a role in blood pressure regulation and hence development of preeclampsia (Sarma & Gambhir, 2005).

Previous studies have evaluated the serum magnesium of pregnant women with the diagnosis of preeclampsia, but these studies did not measure the magnesium level of the pre-eclampsia women since the beginning of their pregnancy. At the moment, evidence to support routine magnesium supplementation for all pregnant women has not been substantiated by research, though most studies have reported reduced magnesium levels in pregnancy and worse levels in preeclampsia (Catov *et al.*, 2009; Harrison, Fawcus, & Jordaan, 2007; Roberts *et al.*, 2010).

In preeclampsia, there is endothelial injury from abnormal invasion of the spiral arteries by the trophoblast mediated by endothelial factors with abnormal vessel formation and permeability (Luppi *et al.*, 2010; Maynard *et al.*, 2003; Muy-Rivera, Vadachkoria, Woelk, & Qiu, 2005; Smith & Kenny, 2006). Interaction between tyrosine kinases and the endothelial growth factors occurs. An increase in the amount of antagonists to endothelial growth factors being expressed by the placenta has been observed in preeclampsia (Maynard *et al.*, 2003; Tsatsaris *et al.*, 2003). Data from endothelial growth factor knockout mice supports genetic evidence that antagonism of endothelial growth factors play a role in the symptomatology of preeclampsia (Eremina *et al.*, 2003).

It can be seen clearly that the pathogenesis of preeclampsia is a syndrome that takes its root from abnormal placentation and therefore it is not surprising why it is terminated by termination of pregnancy. The pathogenesis of preeclampsia is theorised into three main factors namely defective placentation, placental ischaemia and endothelial cell dysfunction (Roberts & Lain, 2002).

Inhibition of platelet aggregation and adhesion to vascular surfaces is mediated by a vasodilator named nitric oxide (NO). Endothelial cell dysfunction and abnormalities in NO production has been observed in preeclampsia, but this observation has not been consistent (Davigde, Stranko, & Roberts, 1996; Nobunaga *et al.*, 1996; Seligman, Buyon, Clancy, Young, & Abramson,

1994). Nevertheless, factors increasing nitric oxide levels have been postulated to prevent the development of preeclampsia. High levels of NO metabolites have been reported in fetoplacental circulation (Ramsay, Sooranna, & Johnson, 1996). Altered production of NO may influence reduced placental blood flow associated with preeclampsia. Vascular endothelial growth factors stimulate angiogenesis and also promote NO and prostacyclin formation, signalling molecules that are reduced in preeclampsia (He *et al.*, 1999). It has been noted that a high percentage of cancer patients who receive vascular endothelial growth factor antagonists develop proteinuria and hypertension (Kabbinavar *et al.*, 2003).

In normal pregnancy, there is an increase in cardiac output and intravascular circulatory volume. Even though these changes occur, in normal pregnancy blood pressure levels decrease as a result of decreased peripheral vascular resistance secondary to marked systemic vasodilatation (De Swiet, 1988; Grindheim, Estensen, Langesaeter, Rosseland, & Toska, 2012; Sanghavi & Rutherford, 2014). Gestational vasodilatation is mostly affected by NO (Sladek, Magness, & Conrad, 1997). The reduction in blood pressure in normal pregnancy is compensated by a rise in heart rate (Carruth, Mirvis, Brogan, & Wenger, 1981; Clapp & Capeless, 1997; Grindheim *et al.*, 2012; Mahendru, Everett, Wilkinson, Lees, & McEniery, 2014). The increased heart rate persists throughout pregnancy so as to optimise cardiac output (Mahendru *et al.*, 2014; Soma-Pillay, Nelson-Piercy, Tolppanen, & Mebazaa, 2016).

While most researches comparing serum electrolytes and NO levels in normal and preeclamptic pregnancy have occurred in developed nations, there has been a paucity of literature in developing nations where most of the maternal deaths from preeclampsia occur (Cunningham, Veno, & Bloom, 2010; Duley, 2009; Shennah, 2007).

Since serum electrolytes and NO are critical physiological agents and their deficiency might contribute to the development of preeclampsia and its complications, it is necessary to

undertake a research, evaluating the relationship between major serum electrolytes and NO, and the risk of preeclampsia in Ghanaian women.

1.2 Problem Statement

Preeclampsia/eclampsia still remains as one of the major causes of maternal and foetal morbidity and mortality worldwide especially developing countries including Ghana (Centre for Maternal and Child Enquiries (CMACE), 2011; Duley, 2009; Steegers, von Dadelszen, Duvekot, & Pijnenborg, 2010). In spite of intensive research, the precise cause of preeclampsia is not known with certainty (Diejomaoh *et al.*, 2004). The cause of placental dysregulation, generalised systemic vasoconstriction and other clinical features characterising preeclampsia with consequent maternal and foetal morbidity are poorly understood (Duley, 2009; Urato & Norwitz, 2011).

There is paucity in literature in Ghana concerning the role of major serum electrolytes and nitric oxide levels of pregnant women in the development of preeclampsia and eclampsia to the best of my knowledge. Although some studies have reported that calcium and magnesium have a relevant effect on the blood vessels of pregnant women (Skjærven, Wilcox, & Lie, 2002), other studies have reported conflicting results concerning serum sodium and potassium levels in women with pregnancy induced hypertension and preeclampsia compared with normotensive pregnant women (Yussif, Salih, Sami, & Mossa, 2009). However, all these electrolytes appear to be risk factors or predisposing factors to preeclampsia. No general agreement seems to have been reached yet on the role electrolytes play in the pathophysiology of preeclampsia. There is also conflicting literature as to whether the alteration in the function of the endothelium seen in preeclampsia results in a pathophysiologic decrease in NO synthesis (Buhimschi, Saade, Chwalisz, & Garfield, 1998; Muy-Rivera *et al.*, 2005). Notwithstanding above controversies, systematic reviews and meta-analyses has shown that preeclamptic women have significant increased risk of incidence of cardiovascular diseases, obesity,

diabetes and insulin resistance later in life (Bellamy, Casas, Hingorani, & Williams, 2007; Seely & Solomon, 2003; Smith, Pell, & Walsh, 2001). Thus preeclampsia has a huge global and economic burden. There is therefore the need to carry out this study to find out the role these electrolytes and NO play in the pathophysiology of preeclampsia in Ghanaian women.

1.3 Justification/Relevance

Mineral deficiencies have been postulated as a cause of preeclampsia in developing countries. This association has however, been found to have regional variation (Adam, Malatyalioglu, Alvur, & Talu, 2001; Cunningham *et al.*, 2005; Joshi, Sapre, & Govilla, 2003). Altered NO production has also been postulated to be associated with preeclampsia even though the results are conflicting. Confirmation of these findings will be necessary in developing strategies for managing preeclampsia as there are no reliable cost effective screening tests and well established primary prevention measures.

Currently in Ghana clinicians use magnesium to treat pregnancy related complications such as preterm labour, severe preeclampsia and eclampsia without monitoring the serum magnesium levels of these patients. This could be deleterious because both hypomagnesaemia and hypermagnesaemia has its antecedent complications. It would therefore be helpful to study the levels of these markers during pregnancy and in preeclamptics in Ghana to guide clinical practice. The estimation of serum electrolytes in preeclampsia provides a very useful index for the study of physiological and pathological changes during pregnancy.

This study will therefore serve as an informed basis for identification of avenues and strategies for a more effective approach aimed at reducing some of the cardiovascular complications in pregnancy related to these biochemical indices.

1.4 General Aim

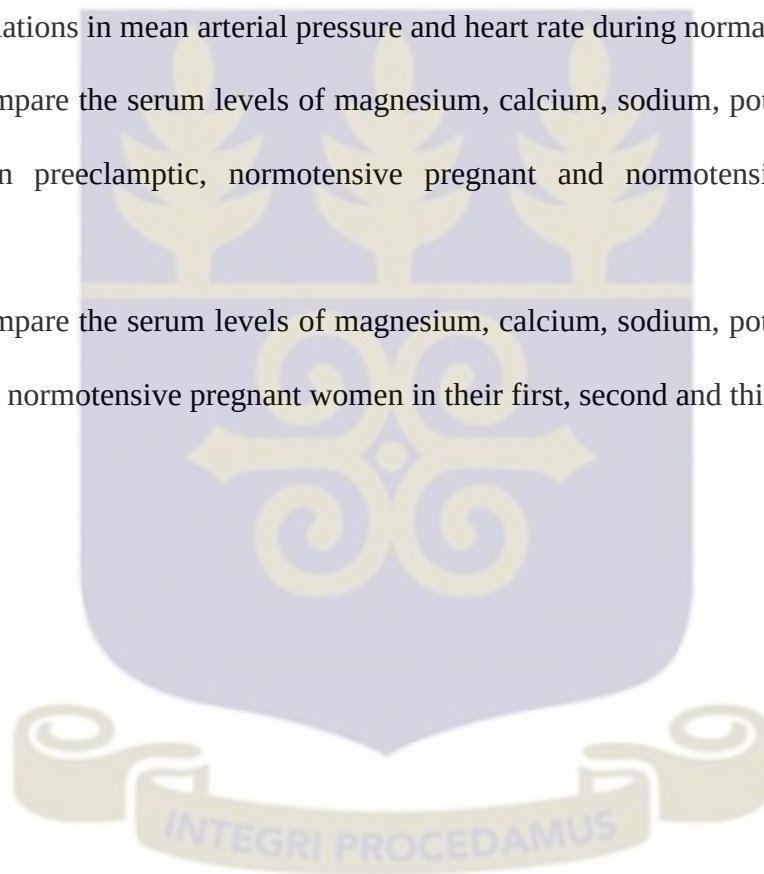
The aim of this study was to determine the electrolyte physiology of selected serum electrolytes and nitric oxide in preeclampsia at the Korle-Bu Teaching Hospital.

1.5 Objectives

The specific objectives of the study were:

To determine:

1. The variations in mean arterial pressure and heart rate during normal pregnancy.
2. And compare the serum levels of magnesium, calcium, sodium, potassium and nitric oxide in preeclamptic, normotensive pregnant and normotensive non-pregnant women.
3. And compare the serum levels of magnesium, calcium, sodium, potassium and nitric oxide in normotensive pregnant women in their first, second and third trimester.



CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Pre-eclampsia

Preeclampsia is a disorder of widespread vascular endothelial malfunction and vasospasm, which occurs after gestational age of 20 weeks and can present as late as 4-6 weeks postpartum (Tavana & Hosseinmirzaei, 2013).

Preeclampsia is generally believed to be an endothelial cell disorder leading to a mild-to-severe microangiopathy of target organs such as the liver, kidney, brain and placenta. Though hypertension is the most common sign of preeclampsia, it may not be the most earliest pathogenic sign as it is evident that other organ involvement before hypertension becomes fulminant is common. The endothelial dysfunction seen in preeclampsia is attributed to circulatory maladaptation, inflammation, humoral, mineral, oxidative stress and metabolic abnormalities.

Preeclampsia was formerly believed to be self-limiting resolving with delivery of the placenta with no long term complications in the mother. However, current evidence suggests that the endothelial dysfunction seen in preeclamptic pregnant women may persist years after the episode and therefore preeclamptic women may be at high risk of cardiovascular diseases later in life (Mutter & Karumanchi, 2008). It has been noted that preeclamptic women show abnormal endothelial dependent flow mediated arterial dilatation three years after the preeclamptic episode (Chambers *et al.*, 2001).

Early detection and prompt management helps in reducing the complications of this condition. Despite its prevalence and severity, the pathophysiology of this multisystem disorder is still

poorly understood and its aetiology has not yet been fully elucidated. Environmental and nutritional factors may play a role in the aetiology of pre-eclampsia.

2.2 Classification of Hypertensive Disorders of Pregnancy

Hypertensive disorders in pregnancy are classified into four main categories namely chronic hypertension, gestational hypertension, preeclampsia (*de novo* or superimposed on chronic hypertension) and white coat hypertension (Tranquilli *et al.*, 2014).

2.2.1 Chronic Hypertension

Chronic hypertension in pregnancy is defined as hypertension predating pregnancy (Tranquilli *et al.*, 2014). Chronic hypertension in pregnancy may be due to primary hypertension, secondary renal disease (such as reflux nephropathy or glomerulonephritis), fibromuscular hyperplasia of the renal arteries or primary hyperaldosteronism. Persistent hypertension even after delivery of the foetus and diagnosed for the first time during pregnancy is also classified as chronic hypertension.

2.2.2 Gestational Hypertension

Gestational hypertension refers to the *de novo* occurrence of hypertension after 20 weeks of gestation, in the absence of the abnormalities that characterise preeclampsia (Tranquilli *et al.*, 2014). It is benign but can progress to pre-eclampsia in about 25% of cases, especially when the high blood pressure occurs before 32 weeks of gestation (Saudan, Brown, Buddle, & Jones, 1998). If the patient's blood pressure returns to normal by 12 weeks postpartum, a diagnosis of transient hypertension of pregnancy is assigned. However, with persistent elevation of blood pressure after 12 weeks postpartum, a diagnosis of chronic hypertension is assigned. Therefore, gestational hypertension is only used transiently in pregnancy until definitive diagnosis is assigned postpartum.

2.2.3 Preeclampsia

Preeclampsia is defined as the onset of hypertension after 20 weeks of gestation with the coexistence of one or more of the following new-onset conditions (Tranquilli *et al.*, 2014) as described in page 1 of introduction.

The presence of oedema is not included in the definition because it is nonspecific in pregnancy. Preeclampsia has been grouped into two disease entities by various researchers namely early-onset preeclampsia and late-onset preeclampsia.

2.2.3.1 Early and late-onset Preeclampsia

Early-onset preeclampsia is defined by some researchers as preeclampsia developing before 34 weeks of gestation, as compared to late-onset preeclampsia that develops at or after 34 weeks' gestation (Fang, Dawson, Lohsoonthorn, & Williams, 2009; Meyer, Mercer, Friedman, & Sibai, 1994; Raymond & Peterson, 2011). These two disease entities (Sibai, Dekker, & Kupferminc, 2005) are associated with different maternal and foetal outcomes, heritability, clinical features and biochemical markers. It has also been postulated that the mechanism involved in these diseases may be different (Chang, Muglia, & Macones, 2010). Early onset preeclampsia is associated with shallow trophoblastic invasion and impaired vascular remodelling as a result of production of soluble endoglin (sENG) which inhibits extravillous trophoblastic invasion thereby causing abnormal uterine artery Doppler, foetal growth restriction, and adverse maternal and neonatal outcomes (Murphy & Stirrat, 2000; Ness & Sibai, 2006; Saito & Nakashima, 2014). In contrast, late-onset preeclampsia is mostly associated with normal or slight increased uterine resistance index, a low rate of foetal involvement, and more favourable perinatal outcomes (Ness & Sibai, 2006; Sibai *et al.*, 2005; Valensise, Vasapollo, Gagliardi, & Novelli, 2008).

Other researchers also define early-onset preeclampsia as preeclampsia that occurs before 37 weeks of pregnancy as compared to late-onset preeclampsia which is preeclampsia occurring at or after 37 weeks of pregnancy (Obed & Aniteye, 2006) on the basis that pregnancy before 37 weeks is described as preterm whereas pregnancy at or after 37 weeks is described as term. Most preeclampsia occur at or near term with only 10% of cases occurring before 34 weeks of gestation (Lain & Roberts, 2002). Early-onset preeclampsia is more severe compared with late-onset preeclampsia (Chang *et al.*, 2010; Fang *et al.*, 2009), examples include worse perinatal outcomes such as small for gestational age infants, increased incidence of HELLP syndrome, placental abruptio and increase in long term maternal health complications such as cardiovascular risk and metabolic syndrome (Chang *et al.*, 2010).

2.2.3.2 Eclampsia

Eclampsia is the occurrence of seizures of unknown cause in preeclampsia. It may occur in mid-gestation or even postpartum.

2.2.3.3 Superimposed Preeclampsia

Superimposed preeclampsia occurs in the setting of chronic hypertension in the presence of one or more of the above mentioned preeclamptic features (Tranquilli *et al.*, 2014). In the setting of renal disease, other features (such as new onset thrombocytopaenia, neurological features or liver dysfunction) other than impaired GFR or proteinuria is used in the diagnosis. The prognosis of superimposed preeclampsia is worse for both the mother and the foetus as compared to either chronic hypertension in pregnancy or preeclampsia alone (Podymow & August, 2007).

2.2.4 White-coat Hypertension

Elevated blood pressure of patients noted only at clinics or office visits by doctors is described as white-coat hypertension. About 1 out of 4 patients with raised clinic or office blood pressures

have white-coat hypertension (Franklin, Thijs, Hansen, O'Brien, & Staessen, 2013). These recognised blood pressure elevations can be avoided either by having a nurse check the blood pressure of the patient or self-home blood pressure monitoring (Brown, Buddle, & Martin, 2001).

2.3 Epidemiology

Two – eight percent (2-8%) of all pregnancies worldwide are complicated by preeclampsia causing over 63000 maternal deaths annually (Duley, 2009; World Health Organisation, 2005). Maternal mortality rate of preeclampsia is highest in low and middle income countries. However, preeclampsia is still a life threatening disorder even in developed countries (Khan *et al.*, 2006). There is a five-fold increase in perinatal deaths from prematurity as a result of preeclampsia (Lain & Roberts, 2002). Preterm birth in itself is responsible for the majority of neonatal deaths and nearly one half of all cases of congenital neurologic disability (Goldenberg & Rouse, 1998). Fifteen percent (15%) of all premature deliveries in the United States (US) are as a result of preeclampsia (Lain & Roberts, 2002). Another risk for the infants is intrauterine growth restriction.

Preeclamptic women have been observed to have a four-fold increase in risk of having a small for age baby compared to normotensive pregnancies. Early onset preeclampsia causes 53% incidence of small for age infants during delivery (Ødegård, Vatten, Nilsen, Salvesen, & Austgulen, 2000). Small for gestational age infants born to preeclamptic mothers are known to have a high incidence of diabetes, hypertension and coronary heart diseases later in their adulthood (Barker, 2004). In the same manner, preeclamptic women are also known to have a high risk of peripheral vascular diseases, cerebrovascular diseases, cardiac diseases and cardiovascular mortality later in life (McDonald, Malinowski, Zhou, Yusuf, & Devereaux, 2008). Ischaemic heart diseases later in life has a strong association with recurrent and severe

hypertensive disorders occurring during pregnancy (Wikström, Haglund, Olovsson, & Lindeberg, 2005).

2.4 The Fetoplacental Unit

In pregnancy, the mother, the foetus and the placenta are interdependent and therefore form a functional unit called the feto-placento-maternal unit or the fetoplacental unit. The placenta secretes hormones which controls the movement of other hormones to between the maternal circulation, foetal circulation and the placenta. The fetoplacental unit is an allograft as it contains paternal antigens, foreign to its maternal host hence normal pregnancy may produce immunologic adaptive mechanism. The human placenta is divided into three layers namely the basal plate (maternal surface), the terminal villous unit and the chorionic plate (foetal surface). The basal plate anchors with maternal endometrium. The terminal villus is the site for nutrient and gas exchange. The chorionic plate contains the foetal vessels (Furuya, Ishida, Aoki, & Fukamizu, 2008). The chorionic plate is covered by the amnion and the chorionic membranes. The umbilical cord takes chorionic veins and arteries on the chorionic plate at its centre. The fundamental structure of the placenta is formed during the first half of gestation (Reynolds *et al.*, 2005). There are three trophoblastic layers between the maternal and foetal circulatory interface (Adamson *et al.*, 2002; Georgiades, Ferguson-Smith, & Burton, 2002; Rossant & Cross, 2001).

The placenta increases in weight almost twice during the last stage of its formation (Cunningham *et al.*, 2005) with an increase in its functional capacity (Furuya *et al.*, 2008; Reynolds *et al.*, 2005).

In preeclampsia there is a disturbance in terminal villus differentiation leading to redistribution of foetal body blood flow to most critical organs such as the brain and the heart under these hypoxic conditions further reducing placental blood flow and increasing vascular resistance.

2.5 Pathophysiology of Pre-eclampsia

The aetiology of preeclampsia lies in the placenta though it remains unknown (Smith & Kenny, 2006; Uzan, Carbonnel, Piconne, Asmar, & Ayoubi, 2011). Preeclampsia is known to occur only in the presence of a placenta as in for example molar pregnancy and resolves after its delivery. Placental growth is a regulated process and it is vital for normal foetal development and for maintenance of successful pregnancy. Normal placental development requires that cytotrophoblast invades the maternal spiral arterioles (Chesley *et al.*, 1976; Smith & Kenny, 2006) as shown in figure 2.1.

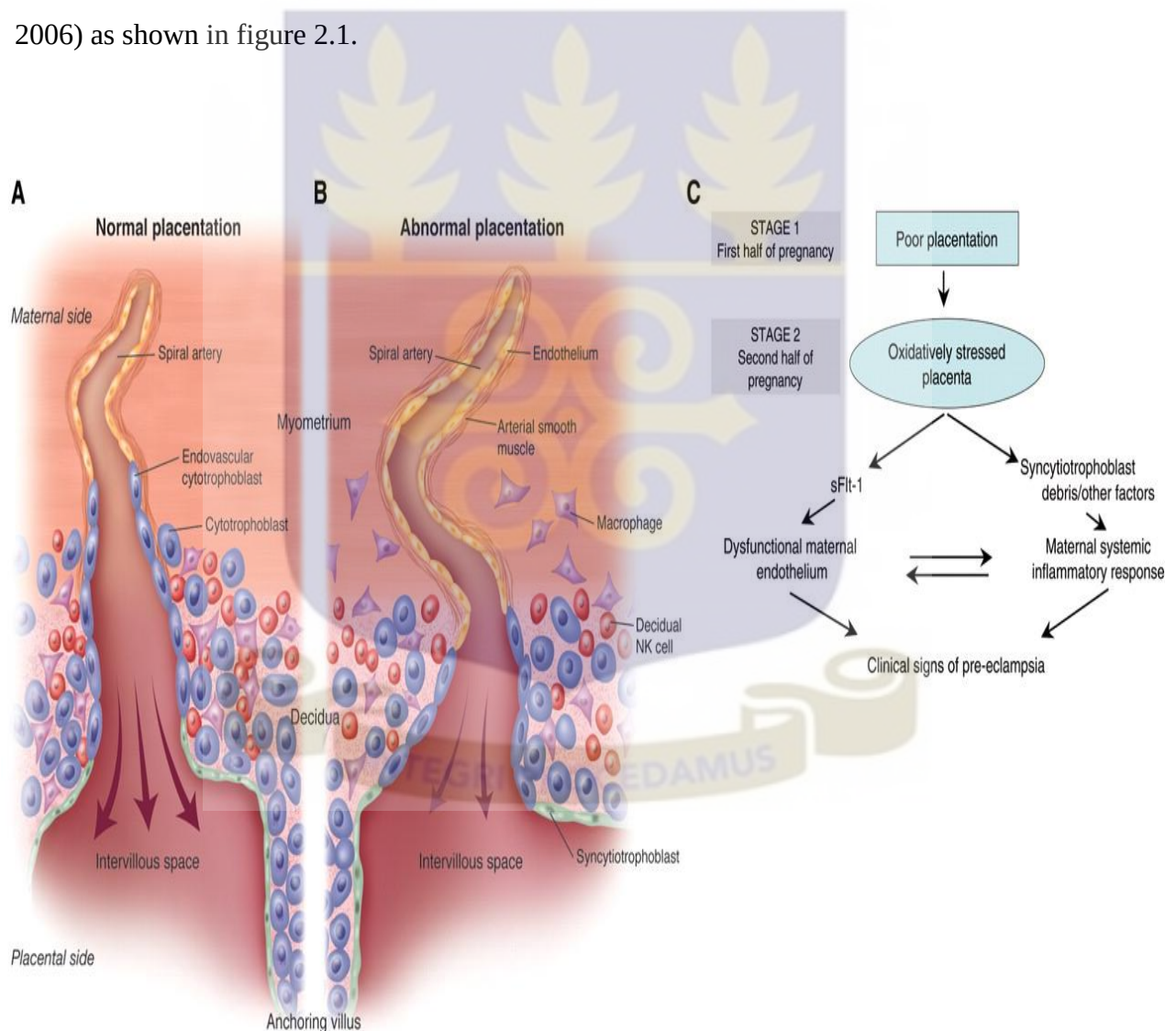


Figure 2.1: A figure showing normal (A) placentation in normal pregnancy compared to abnormal (B) placentation in preeclampsia.

Source: <http://placentas.wordpress.com/implantation> (2011)

2.5.1 Normal Placentation

Extensive angiogenesis occurs in normal placentation so as to facilitate optimal blood supply to the placenta and the foetus with foetal derived extravillous trophoblast invading the uterine arteries and converting the epithelial phenotype to a more endothelial phenotype and therefore making a direct contact with the maternal blood (George & Granger, 2010; Uzan *et al.*, 2011). The spiral arteries are therefore remodelled into low resistance, large capacitance vessels. This usually occurs at the latter part of the first trimester and ends by 20 weeks of gestation. The spiral arteries are branches of the uterine artery and they provide the necessary blood supply to the placenta.

The developing placenta's cytotrophoblast invades the uterine wall at the early stages of pregnancy, interfering with the endothelium and tunica media of the spiral arteries causing a remodelling of the spiral arterial wall leading to conversion of the spiral arteries from highly resistant low flow vessels into low resistance high flow vessels which are needed for sustenance of normal placental development (Agarwal & Karumanchi, 2011).

Expression of certain molecules occurs during trophoblastic invasion. These molecules are adhesion molecules, metalloproteinases, extracellular matrix molecules, Class 1b major histocompatibility complex molecules, and histocompatibility leukocyte antigen. These structural changes with expression of the above molecules leads to the transformation from epithelial nature to endothelial phenotype (Agarwal & Karumanchi, 2011).

There is an impairment of cytotrophoblastic invasion of the myometrial portion of the spiral arteries in preeclampsia leading to narrowing of the spiral arteries with limited blood supply to the foetus (George & Granger, 2010). This impairment becomes significant as the pregnancy progresses as a result of an increase in foetal demand of nutrients for growth secondary to limited amount of blood flow in the spiral arteries. This eventually causes placental ischaemia

and microinfarction with subsequent release of placental factors leading to an imbalance in angiogenic factors and therefore widespread endothelial dysfunction that is seen in preeclampsia (Lee *et al.*, 2007).

2.5.2 Abnormal Placentation

The pathophysiology of preeclampsia is not well understood however a two-step model that is widely accepted has been described (Roberts & Hubel, 2009). The physiological change during a normal pregnancy involves spiral artery remodelling. Trophoblasts invade and replace the arteries' endothelial cells and induce arterial smooth muscle cell apoptosis, resulting in large non-vasoactive vessels (Pijnenborg, Dixon, Robertson, & Brosens, 1980).

The first step of the model involves morula to blastocyst differentiation anomalies or trophoblast to cyto/syncytio-trophoblast differentiation anomalies causing abnormal spiral artery invasion by the trophoblast. The result is an inadequate placentation, followed by intermittent perfusion of the intervillous space and fluctuating levels of oxygen. This causes an increased oxidative stress evident by generalised oxidative modification of lipids and proteins in the placenta as a result of hypoxic-reoxygenation damage. Tissue apoptosis and necrosis also occurs affecting placental formation via regulation by placental growth factors and syncytin. There is an increase in placental volume with alteration of the shed debris STMP (syncytiotrophoblast microparticles) into maternal circulation as a result of the above haemodynamic changes (James, Whitley, & Cartwright, 2010).

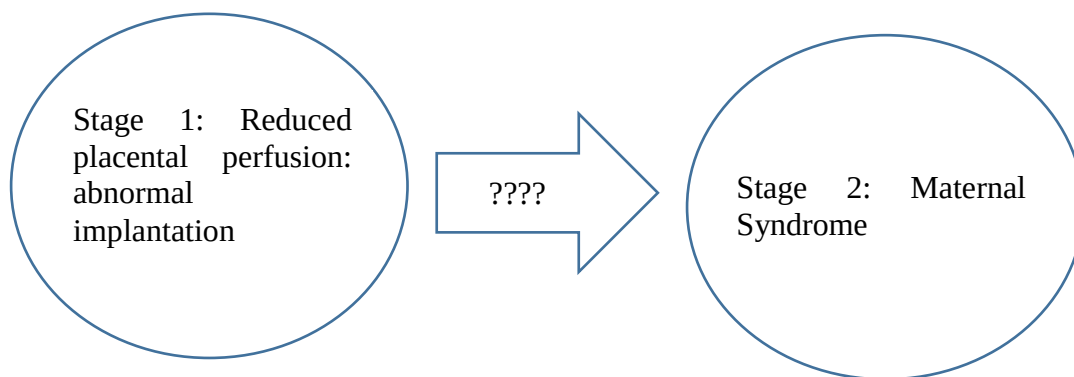
There is an evident general systemic inflammatory response of the endothelial system during the second step of the model (Roberts *et al.*, 1989). The induced oxidative stress sets up a positive feedback mechanism worsening the inflammatory response (Redman & Sargent, 2009). Syncytiotrophoblast microparticles, cytokines, apoptotic factors and anti-angiogenic factors are among a number of circulating agents identified as mediating endothelial

dysfunction, elevated vascular permeability and oxidative stress in preeclampsia (Redman & Sargent, 2009).

An activation of the coagulation cascade with formation of occlusive microthrombi is also a result of the endothelial dysfunction (Lain & Roberts, 2002). The ability of the maternal system to handle the deficits in placentation and subsequent challenge to the maternal cardiovascular system partly depend on the immune system, as systemic inflammatory stress plays a key role in endothelial cell activation (Redman & Sargent, 2009). Women with a pre-gestational endothelial dysfunction, such as pre-existing hypertension, obesity and dyslipidaemia, have an increased risk of preeclampsia.

The changes that are relevant to the development of preeclampsia can be detected in the first trimester, long before the failed vascular remodelling necessary to reduce placental perfusion occurs (Smith & Kenny, 2006). The two-stage model of preeclampsia (Figure 2.2) was developed to provide a guiding framework for scientists in searching for causation of the disorder as the real aetiology remains unknown. Although it was originally thought that maternal factors interact only with decreased placental perfusion to cause the maternal syndrome, however, it is currently thought that maternal factors may itself be involved in the genesis of the reduction in placental perfusion (Bell, 2010).

Known complications related to pre-eclampsia are eclampsia, abruptio placentae with disseminated intravascular coagulopathy, cerebral haemorrhage, pulmonary oedema, hepatic failure, HELLP (Haemolysis Elevated Liver enzymes, Low Platelet count) syndrome and acute renal failure (Duley, 2009; Sibai, Caritis, Hauth, National Institute of Child Health, & Human Development Maternal-Fetal Medicine Units Network, 2003).



Source: (Roberts & Hubel, 2009)

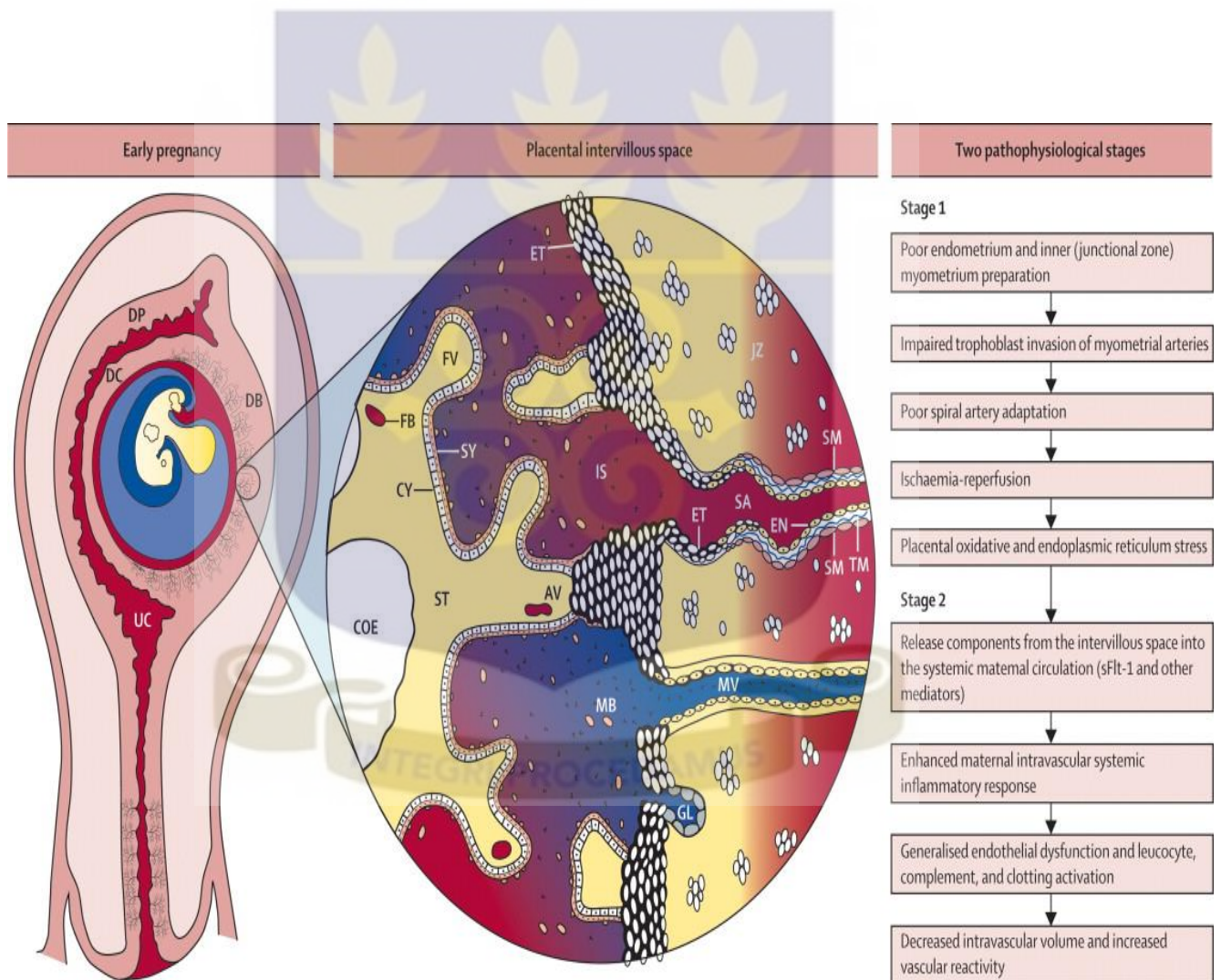


Figure 2.2: The two-stage model of preeclampsia

Source: (Steegers *et al.*, 2010)

2.6 Risk factors of Pre-eclampsia

Risk factors of pre-eclampsia are of multiple origin. Genetic predisposition is a significant factor underlying preeclampsia and the risk of developing the condition is increased two to three fold for women whose mother or sister has had preeclampsia (Smith & Kenny, 2006). Other maternal risk factors for preeclampsia include previous episodes of preeclampsia, black race, diabetes or insulin resistance, thrombophilias, collagen vascular diseases, obesity, multiple gestation, extremes of age (<20years or >40 years) and molar pregnancy (Lain & Roberts, 2002; Obed, Wilson, & Elkins, 1994). The risk of developing superimposed preeclampsia is increased by 15% to 25% in chronic hypertensive patients. (Smith & Kenny, 2006).

Multigravidas with a new partner and primigravidas are also known to have an increased risk to the development of preeclampsia as a result of limited sperm exposure (Uzan *et al.*, 2011). A limited interval between first coitus and conception (coital interval) with the same partner also increases the likelihood of preeclampsia as there is a vivid support for partner specificity in this context. These notable findings suggest that exposure to seminal plasma or paternal sperm or both can cause an immunological tolerance between the mother to fetopaternal alloantigens and hence failure of this immuno-regulation increases one's chances of developing preeclampsia (Redman & Sargent, 2010).

Artificial insemination by means of oocyte donation or embryo donation also increases a woman's risk of developing preeclampsia. However, in contrast, heterologous blood transfusion, previous history of abortion or healthy pregnancy with the same partner, history of cohabitation and history of oral sex with the father of a pregnancy has been reported to reduce one's risk of developing preeclampsia. It has also been noted that men who are currently fathering a preeclamptic pregnancy, have previously fathered a preeclamptic pregnancy either with the same woman or another woman. All these features support a role for paternal antigens

and protection from prior exposure (Taylor, De Groot, Cho, & Lim, 1997). Risk of preeclampsia is decreased with smoking even though smoking is not recommended for pregnant women (Sibai *et al.*, 2005).

Not all studies have reported the same risks (Pabinger, 2009), as various ways have been suggested to categorise the different risk factors (Dekker & Sibai, 2001; Sibai, 2003). One of the strongest risk factors is a previous pregnancy with preeclampsia. The reported recurrence rate of preeclampsia in literature varies with rates up to 65% observed (Sibai, Mercer, & Sarinoglu, 1991). Recurrence rate of preeclampsia is affected by gestational age at onset of preeclampsia, the severity of preeclampsia and presence of comorbid medical, vascular or renal conditions (Dildy, Belfort, & Smulian, 2007). Researches in the past have focussed on trying to identify a single effective screening tool for preeclampsia but this has been unsuccessful as to date no single tool has been successfully used to diagnose and prevent preeclampsia (Jain, Sharma, Kulshreshtha, Mohan, & Singh, 2010; Vasiljević, Vasiljević, & Plečas, 1995).

Dietary deficiency of mineral ions have been implicated in various reproductive age health-related anomalies especially in developing countries (Tavana & Hosseinmirzaei, 2013). During pregnancy, there is an increase in demand for minerals such as calcium and magnesium due to the growing foetus therefore worsening the risk of deficiency (Jain *et al.*, 2010). Changes in levels of these elements could affect pregnancy. One of the problems that may be influenced by nutrient deficiencies is preeclampsia. More emphasis are currently being placed on the association between preeclampsia and maternal serum minerals (Adam *et al.*, 2001; Cunningham *et al.*, 2005). Dietary deficiencies of calcium and magnesium during pregnancy have been implicated in preeclampsia (Catov *et al.*, 2009; Jain *et al.*, 2010; Nygaard, Valbø, Pethick, & Bøhmer, 2008; Roberts *et al.*, 2010). The estimation of serum electrolytes in preeclampsia provides a very useful index for the study of physiological and pathological changes during pregnancy.

2.7 Clinical Features and Diagnosis of Preeclampsia

Clinically preeclampsia presents as new onset of hypertension and proteinuria after 20 weeks of gestation (Grill *et al.*, 2009; Maynard & Karumanchi, 2011). Oedema is no longer considered as part of the diagnostic criteria because it has been considered as common in normal pregnancy with an incidence of 60-80% therefore reducing its positive predictive value. However, sudden facial and hand oedema may be the only earliest presenting sign in some patients. Other clinical complications of preeclampsia include seizures (eclampsia), pulmonary oedema, acute renal failure, haemolysis, thrombocytopaenia and acute liver injury (Kwawukume, 2002). Eclampsia may occur with headaches and hyperreflexia (Kwawukume, 2002).

2.8 The role of major cations in Pre-eclampsia

Several studies have reported that serum calcium and magnesium levels have a vasomotor activity on the blood vessels in pregnancy, (Skjærven *et al.*, 2002) whilst others have reported a varying conclusion on the effects of serum sodium and potassium levels on vasomotor activity during pregnancy (Yussif *et al.*, 2009). Therefore, all these electrolytes seem to play a role in vasomotor activity during pregnancy and may possibly have a role in the pathophysiology of preeclampsia. Literature, however, is yet to reach an agreement on the role these electrolytes play in the development of preeclampsia. There is therefore the need to carry out this study to find out the role sodium, potassium, calcium and magnesium may play in the pathophysiology of preeclampsia in Ghanaian women.

2.8.1 The role of Calcium in Pre-eclampsia

Jain and colleagues (2010) implicated a dietary deficiency of magnesium and calcium in the development of preeclampsia. Smooth muscle contraction and cellular water homeostasis is influenced by serum calcium levels. Changes in plasma calcium levels can cause blood pressure changes. Decreased serum calcium and increased cellular calcium leads to an

increased blood pressure in preeclampsia. The raised cellular calcium levels leads to vascular smooth muscle contraction and hence vasoconstriction with an increased vascular resistance (Sukonpan & Phupong, 2005). Calcium supplementation has been reported to half the risk of development of preeclampsia (Kanagal *et al.*, 2014). Kanagal and colleagues in their study on calcium supplementation in pregnancy recognised that daily calcium supplementation of 1.5 – 2 g prevented the development of preeclampsia in normotensive pregnant women. They also noted a reduced blood pressure in the study group compared to the placebo group. The reduction in blood pressure was far more in pregnant women with high risk of developing preeclampsia and in those with very low baseline serum calcium levels. They also reported an improvement in maternal morbidity and mortality. They therefore concluded that adequate calcium supplementation early in pregnancy can prevent the development of preeclampsia (Kanagal *et al.*, 2014). This conclusion was supported by Hofmeyr and colleagues (2010) in their study on calcium supplementation in pregnant women at the community level.

Various studies have supported the finding that low serum calcium levels predispose normotensive pregnant women to developing preeclampsia (Akhtar, Begum, & Ferdousi, 2011; Indumati, Kodliwadmth, & Sheela, 2011; Kumru *et al.*, 2003; Mohieldein, Dokem, Osman, & Idris, 2007; Punthumapol & Kittichotpanich, 2008).

Other studies, however, have not supported the above finding as they noted a non-significant difference in serum levels of calcium in preeclampsia compared to normotensive pregnant women (Bera *et al.*, 2011; Golmohammad, Amirabi, Yazdian, & Pashapour, 2008; Salari & Eftekhari, 2005; Vahidrodsari *et al.*, 2008). In a prospective study by Levine and colleagues, they also observed that calcium supplementation during pregnancy does not prevent the development of preeclampsia in healthy nulliparous women (Levine *et al.*, 1998).

It can therefore be deduced from above that the serum calcium picture in preeclampsia is still debatable and even though calcium supplementation is advised in pregnancy, as to whether it plays a role in the development of preeclampsia still remains uncertain.

In 1997, the Institute of Medicine (IOM) reduced the dietary reference intake (DRI) for calcium in pregnant women to equal the recommended values for non-pregnant women (Institute of Medicine, 1997). They acknowledged the fact that though there is an increased demand for calcium during pregnancy, this demand can be met by bone resorption. A study by Kalkwarf and colleagues (1996) noted that maternal bone resorption during pregnancy recovers fully approximately 1 year postpartum. The recent IOM report still upheld the previous recommendations for dietary calcium intake during pregnancy (Ross *et al.*, 2011).

2.8.2 The role of Magnesium in Pre-eclampsia

Magnesium acts as a cofactor for various enzyme systems (Barbagallo, Dominguez, Galioto, Pineo, & Belvedere, 2010) and is involved in peripheral vasodilation and neuro-humoral transmission (Sukonpan & Phupong, 2005). Magnesium sulphate is used in the treatment of severe preeclampsia and eclampsia. It can therefore be inferred that alterations in magnesium metabolism can be implicated in preeclampsia (Chacko *et al.*, 2010; Haenni, Johansson, Lind, & Lithell, 2002; Krause, Hanson, & Casanello, 2011; Laurant, Hayoz, Brunner, & Berthelot, 2000; Laurant & Touyz, 2000; Meher & Duley, 2007; Moncada, Palmer, & Higgs, 1991; Northcott & Watts, 2004; Resnick, Laragh, Sealey, & Alderman, 1983; Shechter *et al.*, 2000; Sontia & Touyz, 2007; Sukonpan & Phupong, 2005). Magnesium supplementation has been said to prevent preeclampsia, reduce intrauterine foetal growth retardation and increase birth weight (Makrides & Crowther, 2000).

Roberts and colleagues (2003) suggested a reduced serum magnesium level to play a role in the development of preeclampsia. They made their observation on account of in vitro vascular response to magnesium and improvement in eclamptic fits with treatment of magnesium.

Jain and colleagues and Sukonpan and Phupong found a reduced serum magnesium and calcium levels in preeclampsia compared to normotensive pregnant women hence supporting the view that low serum calcium and magnesium levels may be possible causes of preeclampsia development (Jain *et al.*, 2010; Sukonpan & Phupong, 2005).

A research done in Iran by Golmohammad and Yazdian showed no significant difference in the serum levels of magnesium and calcium in preeclampsia compared to normal pregnant women, (Golmohammad *et al.*, 2008) whilst a study by Punthumapol and Kittichotpanich noted significant low serum calcium levels in severe preeclampsia compared to mild preeclamptics and normotensive pregnant women. However, Punthumapol and Kittichotpanich did not notice any significant difference in the serum magnesium levels among normotensive pregnant women and mild and severe preeclampsia (Punthumapol & Kittichotpanich, 2008).

Kumru and colleagues similarly noted a significant lower serum calcium levels in preeclampsia compared to healthy controls, even though there was no significant difference between the two groups with regards to serum magnesium levels. They however postulated the measurement of these electrolytes as a possible marker of early diagnosis of preeclampsia (Kumru *et al.*, 2003).

Akhtar and colleagues in a study recommended early supplementation of calcium as a preventive measure against preeclampsia as they observed a significantly reduced serum calcium levels in preeclampsia (Akhtar *et al.*, 2011).

Various studies have noted significantly reduced levels of serum magnesium levels in preeclampsia compared to normotensive pregnant women (Akinloye, Oyewale, & Oguntibeju, 2013; Catov *et al.*, 2009; Jain *et al.*, 2010; Nygaard *et al.*, 2008; Roberts *et al.*, 2010).

2.8.3 The role of Serum sodium and potassium in Pre-eclampsia

Serum sodium levels has been observed to be reduced in both preeclampsia and pregnancy induced hypertension as compared to normotensive pregnant and non-pregnant women (Indumati *et al.*, 2011; Pitkin, 1972; Ravid, Massarwa, Biron-Shental, & Fejgin, 2005; Searcy, 1969; Tarik & Ward, 2011). There is a reduced intrarenal production of cyclic GMP (cGMP), endothelin and prostaglandin E2 (PGE2) with resultant sodium retention, hypertension and thrombosis in preeclampsia (Clark, Cotton, Hankins, & Phelan, 1997). In preeclampsia there is alteration in cell membrane sodium transport leading to extravascular accumulation of sodium with a reduced plasma sodium level (Clark *et al.*, 1997; Searcy, 1969). However, other studies found no significant change in serum sodium levels of preeclamptic women compared to normal pregnant women (Adewolu, 2013; Bera *et al.*, 2011; Rizk, 1997; Yussif *et al.*, 2009). Caughey and colleagues also noted an increased sodium levels in preeclampsia compared to normotensive pregnant women (Caughey, Stotland, Washington, & Escobar, 2005).

Various studies have reported a statistically non-significant change in the serum potassium levels in preeclampsia and hypertensive pregnant patients compared to normotensive pregnant women (Adewolu, 2013; Bera *et al.*, 2011; Clark *et al.*, 1997; Siddiqui & Rana, 1993; Singh, Dighe, Singh, & Othman, 1993).

However, Yussif and colleagues observed a significantly reduced serum potassium level in hypertensive pregnant women compared with normal pregnant women and proposed a raised serum sodium and a reduced serum potassium level as a pathologic cause of hypertension in pregnancy (Yussif *et al.*, 2009). Yussif and colleagues concluded that a low potassium diet combined with a normal sodium diet can cause sodium retention and therefore development of hypertension (Yussif *et al.*, 2009). Handwerker and colleagues however noted an elevated serum potassium level in preeclampsia compared to normal pregnant women (Handwerker, Altura, & Altura, 1995).

It is obvious from above that the electrolyte picture in preeclampsia may vary in different populations and environments.

2.9 The role of nitric oxide in Pre-eclampsia

In normal pregnancy there are anatomical and functional changes of the cardiovascular system so as to adequately maintain the demands of the pregnancy. These changes include expansion of the total circulating blood volume, an increase in the heart rate and the stroke volume and therefore an increase in the cardiac output. Blood pressure however, reduces progressively until at 20-24 weeks and thereafter increases progressively towards term (VanWijk, Kublickiene, Boer, & VanBavel, 2000).

Therefore, nitric oxide (NO) a potent vascular endothelial relaxant's deficiency can be involved in preeclampsia. Various animal models in which NO synthesis has been inhibited have been associated with symptoms such as hypertension, proteinuria, thrombocytopenia and restricted foetal growth (Diejomaoh *et al.*, 2004; Silver *et al.*, 1996). An endothelial form of NO synthase has been localised to the syncytiotrophoblast and villous endometrium in term pregnancies (Buttery *et al.*, 1994; Conrad & Davis, 1995). The placenta is therefore an important source of NO during pregnancy.

Nitric oxide (NO) is a short-lived radical synthesised from L-arginine. Nitric oxide is implied in endothelial relaxation by activation of guanylate cyclase increasing intracellular levels of cyclic GMP or acting directly on cysteine or tyrosine residues to induce nitrosylation and nitration respectively (Meher & Duley, 2007; Moncada & Higgs, 2006; Moncada *et al.*, 1991).

The main placental vasodilator is nitric oxide and it regulates placental vascular resistance and reactivity, apoptosis and invasion by trophoblast, and aggregation and adhesion of platelets in the placental bed (Krause *et al.*, 2011; Myatt, 2006; Reynolds *et al.*, 2010).

Development of the vascular system of the placenta is important for normal foetal growth (Krause *et al.*, 2011; Myatt, 2006). Placental vascular abnormalities have been associated with placental insufficiencies seen in intrauterine growth retardation, preeclampsia and gestational diabetes mellitus (Reynolds *et al.*, 2006). Recent authors (Meher & Duley, 2007) suggest a role of NO in the control of placental vascular tone and therefore a defect in the L-arginine-NO system is postulated as a possibility in the pathophysiology of preeclampsia. The levels of NO metabolites in maternal serum, urine and cord blood have been noted to be increased, reduced or unchanged in preeclamptic women (Yallampalli & Garfield, 1993). Numerous studies holds the view that preeclampsia is a multisystem disorder with vascular endothelial dysfunction (Roberts *et al.*, 1989), however, as to whether the change in the function of the endothelium noted in preeclampsia results in a decrease in endothelial NO synthesis or its effects is still debatable (Diejomaoh *et al.*, 2004).

The underlying pathology of preeclampsia is presumably because of a relatively hypoxic or ischemic placenta. This usually induces a marked oxidative stress. There is evidence that repeated exposure of the placenta to repeated episodes of ischaemia-reperfusion associated with oxidative stress and increase in xanthine oxidase activity is seen in preeclampsia (Burton & Jauniaux, 2011). Various placental factors have been implicated in this phenomenon including proinflammatory cytokines, angiogenic factors and microparticulate apoptotic debris (Redman & Sargent, 2009). Formerly it was believed that the placenta was mainly poorly perfused (chronically hypoxic placental blood supply) but current evidence shows that there is an intermittent perfusion of the intervillous space leading to hypoxia-reoxygenation or ischaemia-reperfusion injury (Smith & Kenny, 2006).

In normal pregnancy there is a moderate oxidative stress evidenced by an increased circulating levels of low density lipoproteins and decreased total antioxidant levels compared with non-pregnant women. There is also a systemic inflammatory response in normal pregnancy

evidenced by activation of peripheral granulocytes, monocytes and lymphocytes during the third trimester with resultant production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Burton & Jauniaux, 2011; Kossenjans, Eis, Sahay, Brockman, & Myatt, 2000; Myatt *et al.*, 1997; Noris *et al.*, 2004; Redman & Sargent, 2003). Oxidative stress and systemic inflammatory response occurs to a higher degree in preeclampsia evident by such a high increase in protein carbonyls, lipid peroxides, nitrotyrosine residues and DNA oxidation (Burton & Jauniaux, 2011; Myatt & Cui, 2004). This imbalance may be influenced by availability of NO (Meher & Duley, 2007). Preeclampsia is a disorder unique to pregnancy characterized by maternal hypertension, proteinuria, and oedema.

There is failure of adoption of a typical vascular adhesion phenotype by cytotrophoblast in preeclampsia thereby impairing, at the maternal-foetal interphase, blood flow. Nitric oxide is important for cytotrophoblastic invasion and therefore normal placentation. Recent evidence supports alteration in nitric oxide synthesis in the preeclamptic placenta (Lowe, 2000). With respect to this, a decreased placental nitric oxide synthesis has been postulated to explain placental perfusion abnormalities seen in preeclampsia (Baylis, Beinder, Sütö, & August, 1998).

Literature has reported inconsistent results as far as serum nitric oxide levels in preeclampsia compared to normotensive women is concerned. Various studies have reported raised serum nitric oxide levels (Davidge *et al.*, 1996; Nobunaga *et al.*, 1996; Norris, Higgins, Darling, Walshe, & Bonnar, 1999; Smáráson, Allman, Young, & Redman, 1997) whilst others have reported non-significant change, (Conrad & Mosher, 1995; Curtis *et al.*, 1995; Di Iorio, Marinoni, Emiliani, Villaccio, & Cosmi, 1998; Lyall, Young, & Greer, 1995; Monaghan, 1999) and others too, a reduced serum level of nitric oxide (Diejomaoh *et al.*, 2004; Garmendia, Gutiérrez, Blanca, Bianco, & De Sanctis, 1997; Seligman *et al.*, 1994; Silver *et al.*, 1996) in preeclampsia compared to normotensive pregnant women.

In women with high risk of preeclampsia, there has been an observed raised asymmetric dimethyl arginine (ADMA) which may be linked to the development of preeclampsia (Savvidou *et al.*, 2003). Savvidou and colleagues noted uterine artery notches and brachial arterial flow impairment in women with late onset preeclampsia (Savvidou *et al.*, 2003).

Nitric oxide regulates placental blood flow and actively participates in trophoblast invasion and placental development in pregnancy. Magnesium modulates the cardiovascular effect of sodium and potassium and it is a co-factor for the sodium- potassium ATPase activity (Ambwani, Desai, Girdhar, Shah, & Mathur, 1999). Since the electrolytes: calcium, magnesium, sodium and potassium contribute significantly in the functioning of the vascular smooth muscles which plays a key role in the pathogenesis of pre-eclampsia, the present study is designed to evaluate the role of these electrolytes and NO in Ghanaian women with preeclampsia.

2.10 Prevention of Preeclampsia

Knowledge on the pathophysiology of preeclampsia and predisposing factors is one major way of preventing the onset of the disease in obstetrics. It is known that changes in various markers actually precede the onset of clinical features of preeclampsia. Currently, risk assessment of preeclampsia development still remains a challenge despite intensive research in this area (Uzan *et al.*, 2011). Identifying women at risk will allow for proper supervision during pregnancy or at latter stages of the pregnancy (Carty, Delles, & Dominiczak, 2008). Identifying markers that can be used as risk assessment in preeclampsia is of vital importance as this can be used to predict, diagnose and development of potential strategies for management of preeclampsia. It can be seen from above literature that a single marker cannot be used for this purpose. Therefore, a combination of markers (biochemical and or biophysical) may be needed to comprehensively predict and prevent preeclampsia.

Biophysical markers have been used to predict the risk of developing preeclampsia. However, these risk factors are non-modifiable and therefore their use is limited (Carty *et al.*, 2008; Chang *et al.*, 2010). Various inconsistent screening tests for preeclampsia have been described in literature (Conde-Agudelo, Villar, & Lindheimer, 2004). Uteroplacental Doppler ultrasound is the commonest and widely used of these screening tests (Carty *et al.*, 2008; Uzan *et al.*, 2011). However, uteroplacental Doppler ultrasound can give excessive variability during the first or second trimester and therefore of limited use in low risk populations (Maynard *et al.*, 2003). It has been noted that in both low and high risk patient groups the positive predictive value of uteroplacental Doppler ultrasound is not high enough to recommend its routine use as a screening tool (Conde-Agudelo *et al.*, 2004).

The main basis of primary prevention of preeclampsia is the detection of modifiable risk factors i.e. maternal placental and endothelial dysfunction markers. Several risk factors have been elucidated for preeclampsia as stated earlier. However, these may not predict preeclampsia by themselves effectively. Numerous laboratory and clinical predictive tests have also been elucidated but these lack specificity and sensitivity (Conde-Agudelo *et al.*, 2004; Uzan *et al.*, 2011).

No single marker has been identified as effectively predicting the risk of preeclampsia in clinical practice and therefore a combination of markers is recommended (Ahmed, 2011; Uzan *et al.*, 2011).

CHAPTER THREE

3.0 METHODOLOGY

3.1 Study Design

This was a case-control cross sectional study conducted at the Obstetrics and Gynaecology clinic of Korle- Bu Teaching Hospital. Normotensive non-pregnant women, normotensive pregnant women and preeclamptic women were recruited consecutively and included in the study.

3.2 Study Site

The study was conducted at the obstetrics and gynaecology clinic of the Korle- Bu Teaching Hospital in Accra from February to May 2016. The Korle-Bu Teaching Hospital is the premiere Teaching Hospital in Ghana. It is the largest tertiary hospital in Ghana and is affiliated with the University of Ghana School of Medicine and Dentistry. The hospital has a 2000 bed capacity. The obstetrics and gynaecology department of the hospital has a 350 bed capacity with 3 operating theatre suites. The department has 65 doctors, 200 nurses and midwives, with a daily antenatal attendance of 100 patients, and a total annual delivery of between 10,000 and 12,000.

Antenatal care at Korle-Bu Teaching Hospital is offered on daily basis from Monday to Friday. During these antenatal visits, the pregnant women are weighed, have their urine tested for proteins and glucose and their blood pressures measured by the attending midwives before being allowed to see doctors in their consulting rooms. At the booking (first) visit, a comprehensive history is taken by the attending physician followed by a thorough physical and obstetric examination.

Pregnant women are usually seen after every four weeks until 28 weeks of gestation, then fortnightly until 36 weeks of gestation and then finally weekly until they deliver. This particular schedule may be modified based on the condition of the pregnant woman and or the pregnancy. This may necessitate more frequent visits than outlined above. Antenatal patients who may have an urgent medical or obstetric condition are usually admitted to the maternity ward for further management.

3.3 Target Population

The study population included normotensive non-pregnant women, normotensive pregnant women and preeclamptic patients attending the obstetrics and gynaecology clinic, Korle-Bu Teaching Hospital between February and May 2016.

3.4 Case Definition

The International Society for the Study of Hypertension in Pregnancy criteria (Tranquilli *et al.*, 2014) was used for the diagnosis of preeclampsia. Therefore, a systolic blood pressure of at least 140mmHg and a diastolic blood pressure of at least 90mmHg measured in the sitting position using a mercury sphygmomanometer at least for two times after a 15 minute rest and in at least 2 consecutive measurements, 6 hours apart, and a proteinuria of 300mg or more in a 24 hour urine collection or $\geq +2$ dipstick on a random urine sample was considered as preeclampsia.

3.5 Inclusion Criteria

The study included normotensive non-pregnant women, women aged between 18 and 40 years. The recruited participants were only those who gave their informed consent to participate in the study.

The recruited subjects were grouped as follows:

1. Normotensive non-pregnant women (control group 1)

2. Normotensive pregnant women with no complications (control group 2)
3. Pregnant women with preeclampsia (cases)

The normotensive pregnant women with no complications (control group 2) were subdivided further into:

1. Normotensive pregnant women in their first trimester of gestation
2. Normotensive pregnant women in their second trimester of gestation
3. Normotensive pregnant women in their third trimester of gestation

3.6 Exclusion Criteria

The following patients were excluded from the study:

1. Pregnant and non-pregnant women who were unwilling or were unable to give their informed consent
2. Pregnant and non-pregnant women on any medical treatment other than iron and folic acid
3. Pregnant and non-pregnant women with chronic hypertension
4. Pregnant and non-pregnant women with a history of kidney disease
5. Pregnant and non-pregnant women with diabetes mellitus
6. Pregnant and non-pregnant women with cardiac diseases
7. Pregnant and non-pregnant women with neuromuscular problems: these were excluded purely based on the presence of muscle weakness, difficulties in walking, nerve palsies and muscular deformities
8. Preeclamptic pregnant women on magnesium sulphate medication
9. Pregnant women with chronic hypertension with super-imposed preeclampsia
10. Non-pregnant women with urinary tract infection, pelvic infections and abnormal uterine bleeding.

3.7 Sampling Technique

The study was conducted at the obstetrics and gynaecology clinic at Korle-Bu Teaching Hospital. The obstetrics department of the hospital offers an antenatal clinic on daily basis from Monday to Friday. At the antenatal clinic, the weight and height of the pregnant women were measured using Inmoclinc mechanical patient weighing scale with height rod (Product: 6003). Their blood pressure and heart rate were then measured using a mercury sphygmomanometer and a stethoscope. Their urine sample was tested for protein and glucose. At their booking visit (first antenatal visit), their detailed obstetric and medical history as well as detailed obstetric and physical examination were obtained and documented in their antenatal book.

The gynaecological department offers a gynaecology clinic on daily basis from Monday to Friday. At the gynaecology clinic, the weight and height of the patients were measured using Inmoclinc mechanical patient weighing scale with height rod (Product: 6003). Their blood pressure and heart rate were then measured using a mercury sphygmomanometer and a stethoscope. Afterwards, patients were ushered into a consulting room where a doctor took their detailed medical and gynaecology history and conducted a detailed physical and gynaecologic examination.

The normotensive non-pregnant women and the normotensive pregnant women in their first trimester were recruited from the gynaecology clinic. These categories of women were sampled consecutively and included in the study after meeting the inclusion/exclusion criteria. An informed consent was obtained from each participant before being recruited into the study.

The normotensive pregnant women in their second and third trimester as well as the preeclamptic women were recruited from the obstetric clinic at the maternity block of the hospital. These categories of women were recruited consecutively and included in the study

after satisfying the inclusion/exclusion criteria. Informed consent was obtained from each participant before being included in the study.

3.8 Sample Size Determination

The serum magnesium level for pregnant women and preeclamptic women is 0.73mmol/L and 0.58mmol/L respectively (Adekanle *et al.*, 2014). The mean difference (d) in serum magnesium levels between pregnant women and preeclamptic women is 0.15mmol/L. A standard deviation (SD) of 0.21 is assumed for the two groups. For a desired power of 80% and a desire level of statistical significance of 5%, Z_{β} of 0.84 and $Z_{\alpha/2}$ of 1.96 were used respectively. Also a ratio (r) of 1 was considered for equal number of controls and cases. The minimum sample size (n) for the study was 30 for each group using the formula (Charan & Biswas, 2013):

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

A total of 162 women were recruited into the study. This consists of 30 preeclamptic women, 100 normotensive pregnant women (control group 1) and 32 normotensive non-pregnant women (control group 2).

3.9 Procedure

3.9.1 Participants demographics

The participants were interviewed by means of a well-structured questionnaire to obtain their demographic, socioeconomic and obstetric characteristics after signing an informed consent form. The information collected included their weight, height, age, parity, gestation and educational status.

3.9.2 Measurement of blood pressure

The blood pressures and heart rates of all participants was measured at the right arm in the sitting position using a mercury sphygmomanometer and a stethoscope at least for two times

after a 15 minute rest and in at least 2 consecutive measurements, 6 hours apart and their respective averages noted for each of the participants.

3.9.3 Collection of blood samples, preparation and storage

The method as described in the Standard Operating Procedure for performing venepuncture in the National Blood Bank (Korle-Bu Teaching Hospital), 2010 was used. Rubber tourniquet was tied to the biceps about 8cm above the elbow joint for less than one minute and the site to be punctured was cleansed with methylated spirit. Then 5mls of blood was drawn from the brachial vein with a 19G hypodermic needle fixed on 5mls syringe. All aseptic conditions were adhered to. Aliquots of 2.5mls of the blood sample from each subject was immediately transferred into a coded Na EDTA and plain test tube. The blood in the Na EDTA tube was mixed in the bottle to prevent clotting by gently inverting the tube four times manually. The blood sample in the plain test tube was then spun at a speed of 2500 rpm for 10mins to separate serum from cells. Serum obtained was stored in a freezer at a temperature of -20°C for later analysis.

3.9.4 Measurement of haematological profile

Full blood count analysis of samples in the Na EDTA tubes was done on the same day of collection using lab system multiskan MS (manufactured by Amisham Bioscience LTD, UK), a three-part auto analyser able to run 19 parameters per sample including haemoglobin concentration, packed cell volume, red blood cell concentration, mean corpuscular haemoglobin, mean cell volume, mean corpuscular haemoglobin concentration, white blood cells and platelet parameters.

3.9.5 Procedures for estimation of serum magnesium, calcium, potassium and sodium levels

Determination of serum magnesium and calcium was done using a Flame Atomic Absorption Spectrometer (Variant 240FS manufactured by VARIAN Australia Pty Ltd). Serum sodium and potassium was analysed using Sherwood Flame Photometer (Model 420 by Sherwood Scientific Ltd). All electrolyte measurements were done at the Chemistry Unit of the Ghana Atomic Energy Commission.

Two (2) grams of serum sample was weighed into a 100ml class A beaker. Twelve (12ml) of concentrated HNO_3 was added to the weighed sample. This was done in a fume chamber. The beaker was then covered with a clean film and placed on a hot plate for a hot plate acid digestion. The sample was digested for 3 hours at a temperature of 45°C . After acid digestion, the sample was transferred into a 50ml measuring cylinder. The sample was then topped up to the 30ml mark with distilled water. The whole content was transferred into a test tube for the electrolyte analysis. Reference standards and blanks were also digested under the same conditions as the samples. These served as internal positive controls. Reference standards that were used are from Fluka Analytical, Sigma-Aldrich Chemie GmbH, a product of Switzerland.

The digested sample was then assayed for the presence of Mg, Ca, K and Na. The digestate was used directly for magnesium and calcium assessment using the Atomic Absorption Spectrometer in an acetylene-air flame. For potassium analysis, 5ml of the digestate was transferred into a 10ml measuring cylinder. Two (2ml) of lithium solution was added to the 5ml digestate and measurement taken. For sodium analysis, a 1:5 dilution of the digested sample was transferred in a 10ml measuring cylinder. Two (2ml) of lithium solution was added to the 1:5 diluted digestate in the measuring cylinder and measurement taken. Sodium and potassium were measured using the Sherwood Flame Photometer (Model 420). All electrolyte determinations were done in duplicates.

3.9.6 Measurement of nitric oxide

Nitric oxide levels were assessed in the plasma samples using the Griess Reagent system (Promega, Madison, USA). The assay relies on a diazotization reaction. The Griess Reagent System is based on the chemical reaction which uses sulfanilamide and *N*-1-naphthylethylenediamine dihydrochloride (NED) under acidic (phosphoric acid) conditions. This system detects Nitrite in a variety of biological and experimental liquid matrices such as plasma, serum, urine and tissue culture medium.

3.9.7 Preparation of a Nitrite Standard Reference Curve

A Nitrite Standard reference curve was prepared for each assay in order to accurately quantify nitrite levels in experimental samples. A volume of 1ml of a 100 μ M nitrite solution was prepared by diluting the provided 0.1M Nitrite Standard (0.1M sodium nitrite in water) 1:1,000 in distilled water. Three columns (16 wells) in the 96-well plate was designated and labelled appropriately for the Nitrite Standard reference curve. A volume 50 μ l of the distilled water was dispensed into the wells in rows with a micropipette. 100 μ l of the 100 μ M nitrite solution was added to the remaining 3 wells in row. Serial two-fold dilutions (50 μ l/well) was done in triplicate to generate the Nitrite Standard reference curve (100, 50, 25, 12.5, 6.25, 3.13 and 1.56 μ M) by plotting the average absorbance value of each concentration of the Nitrite Standard on the Y-axis and the nitrite concentration on the X-axis.

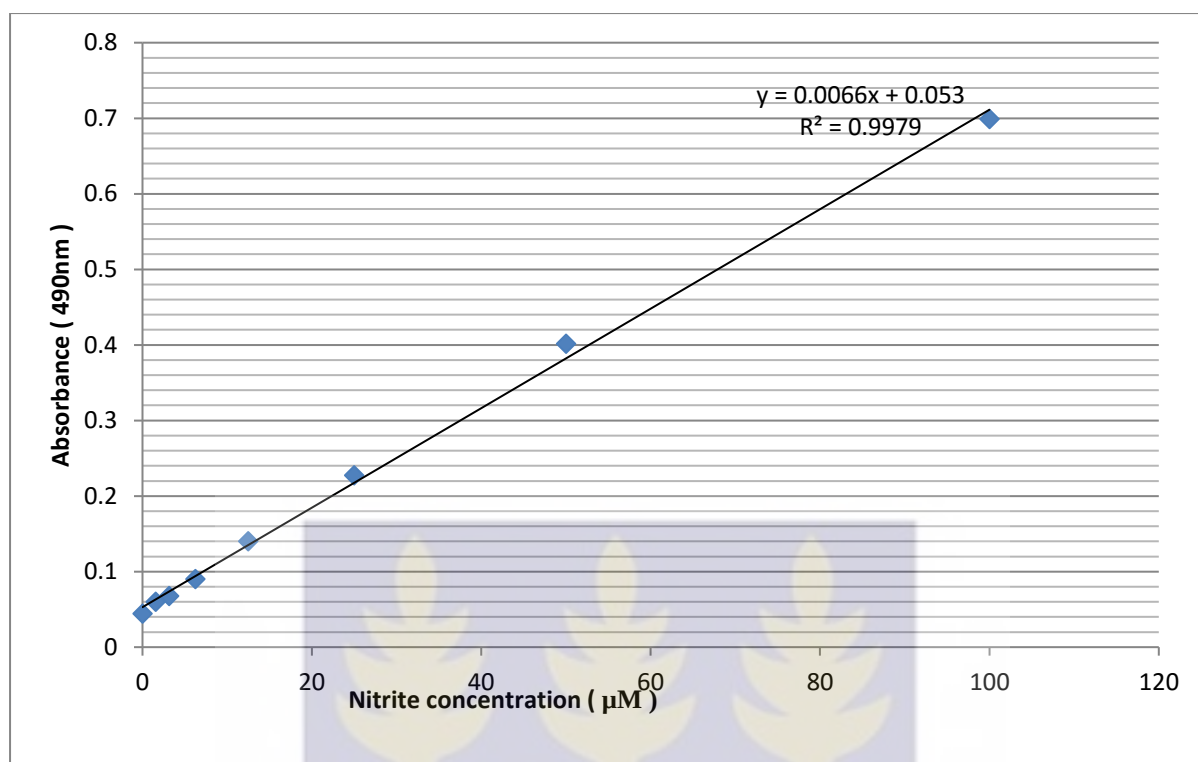


Figure 3.1 Nitrite Standard Reference Curve

From this graph the formula $Y=0.0066x + 0.053$ was derived and was used to calculate the nitrite concentration. Values on the Y-axis are the average absorbances (490nm) and values on the X-axis are the nitrite concentration (µM).

3.9.8 Determination of Nitrite concentration as an index for nitric oxide in experimental samples

Sulfanilamide and N-1-naphthylethylenediamine dihydrochloride (NED) solutions were allowed to equilibrate to room temperature for about 20 minutes. Aliquots of 50µl each of the experimental plasma samples were added to the remaining wells in triplicate and labelled with a marker. Using a multi-channel pipette, 50µl of the sulfanilamide solution was dispensed to all experimental samples and the wells containing the dilution series for the Nitrite Standard reference curve. The plate was then incubated for 10 minutes at room temperature in the dark. After incubation, 50µl of the NED Solution was dispensed to all the wells using a multi-channel pipette. The plate was incubated again for 10 minutes at room temperature in the dark. A

purple/magenta colour started to form immediately. The absorbance was then measured at 490nm using a micro plate reader manufactured by Machine Biotek Instruments, USA (Model EL808) after the incubation period.

The average absorbance value of the triplicates of each sample was determined. The concentration of nitrite as an index for NO in the experimental samples was determined by comparison to the nitrite standard reference curve.

3.10 Data Handling and Statistical analysis

The data collected was entered into Microsoft access database (Microsoft® USA) and analysis was done using statistical package for social science (SPSS®) software version 20.0. The demographic and clinical parameters of the participants such as age, weight, height, BMI and parity were reported as means $\pm$ standard deviations in a tabular form. The laboratory parameters were also presented in means $\pm$ standard deviations in a tabular form. Analysis of variance (ANOVA) was employed to compare the significant difference between preeclampsia, normotensive pregnant and normotensive non-pregnant women as well as the significant difference between 1st, 2nd and 3rd trimester normotensive pregnant women. Where a statistically significant difference was obtained, a follow-up test (post hoc analysis) was done. A p-value less than 0.05 was considered statistically significant.

3.11 Dissemination of results

Findings of this thesis was submitted to the Graduate School, University of Ghana and the Department of Physiology, School of Biomedical and Allied Health Sciences, University of Ghana. Presentations will be made at local and international conferences about the outcome of the study. The final work will be published in a reputable peer reviewed journal.

3.12 Ethical consideration

The study protocol was approved by the Ethical and Protocol Review Committee of the College of Health Sciences of the University of Ghana (2016).

3.13 Consent

Participation in the study was absolutely voluntary. Signed or thumb-printed informed consent was obtained from participants before enrolment into the study (Appendix 1).



CHAPTER FOUR

4.0 RESULTS

4.1 General/Basic Sample Characteristics

A total of 162 women were recruited into the study. This consists of 30 preeclamptic women, 100 normotensive pregnant women (control group 1) and 32 normotensive non-pregnant women (control group 2). The demographic, clinical and basic laboratory characteristics of the recruited women measured are shown in Table 4.1. The means of the various parameters among these 3 categories of women were compared using a one-way analysis of variance (ANOVA) and a p-value of less than 0.05 was adjudged significant at 95% confidence interval.

A significant difference was observed in the mean height, body mass index (BMI), parity, systolic blood pressure, diastolic blood pressure, heart rate, mean arterial pressure (MAP), serum creatinine, serum urea, haemoglobin concentration (HB), haematocrit (HCT), alanine transaminase (ALT), aspartate transaminase (AST), total serum protein and direct serum bilirubin (B direct) among the preeclamptic women, normotensive pregnant women and normotensive non-pregnant women as indicated by their p-values of 0.025, 0.004, 0.009, 0.000, 0.000, 0.001, 0.000, 0.001, 0.000, 0.018, 0.035, 0.001, 0.000, 0.019 and 0.010 respectively. There were however, no significant differences in the mean age, weight, platelet count (PLT) and total serum bilirubin (B total) among the preeclamptic women, normotensive pregnant women and normotensive non-pregnant women as indicated by their p-values of 0.152, 0.094, 0.056 and 0.464 respectively (Table 4.1).

Table 4.1: Demographic, clinical and basic laboratory data for preeclamptic, normotensive pregnant and normotensive non-pregnant women

PARAMETER	PREECLAMPTIC	NORMOTENSIVE PREGNANT	NORMOTENSIVE NON-PREGNANT	P-VALUE
	Mean± SD	Mean ± SD	Mean ± SD	
n	30	100	32	
Age	30.97±5.51	29.63±3.53	31.00±5.11	0.152
Weight	79.40±22.19	71.86±14.73	75.09±17.64	0.094
Height	2.57±0.10	1.61±0.07	1.62±0.05	0.025*
BMI	32.03±7.52	27.53±6.16	28.70±6.23	0.004*
Parity	1.70±1.42	0.94±0.98	1.28±1.59	0.009*
Systolic Blood Pressure	170.13±23.69	113.14±13.44	118.53±16.08	0.000*
Diastolic Blood Pressure	106.30±18.79	68.21±9.07	76.53±9.35	0.000*
Heart Rate	95.80±13.24	85.71±13.70	84.63±13.82	0.001*
MAP	126.20±20.86	82.33±12.49	90.51±9.71	0.000*
Creatinine	73.05±20.74	63.24±13.73	72.17±15.15	0.001*
Urea	2.37±0.83	2.04±0.91	3.26±0.90	0.000*
HB	11.22±1.51	11.07±1.24	11.85±1.50	0.018*
HCT	34.01±4.66	34.49±3.87	35.61±3.65	0.035*
PLT	242.40±55.10	252.56±64.01	281.78±92.28	0.056
GPT/ALT	30.63±8.43	13.53±7.81	19.67±8.73	0.001*
GOT/AST	31.00±6.84	15.39±0.62	16.44±0.74	0.000*
Total Protein	68.13±8.36	68.66±8.50	73.13±7.02	0.019*
B Total	17.80±5.93	16.97±6.88	18.55±5.69	0.464
B Direct	4.28±2.87	3.10±1.70	3.90±2.05	0.010*

* Significant at $p \leq 0.05$; n – Sample size; Mean±SD – Mean plus or minus standard deviation; BMI – Body mass index; MAP – Mean arterial pressure; HB – Haemoglobin; HCT – Haematocrit; PLT – Platelet; GPT/ALT - Glutamate Pyruvate Transaminase/Alanine Transaminase; GOT/AST - Glutamate Oxaloacetate Transaminase/Aspartate Transaminase; B Total – Total serum bilirubin; B Direct – Direct serum bilirubin

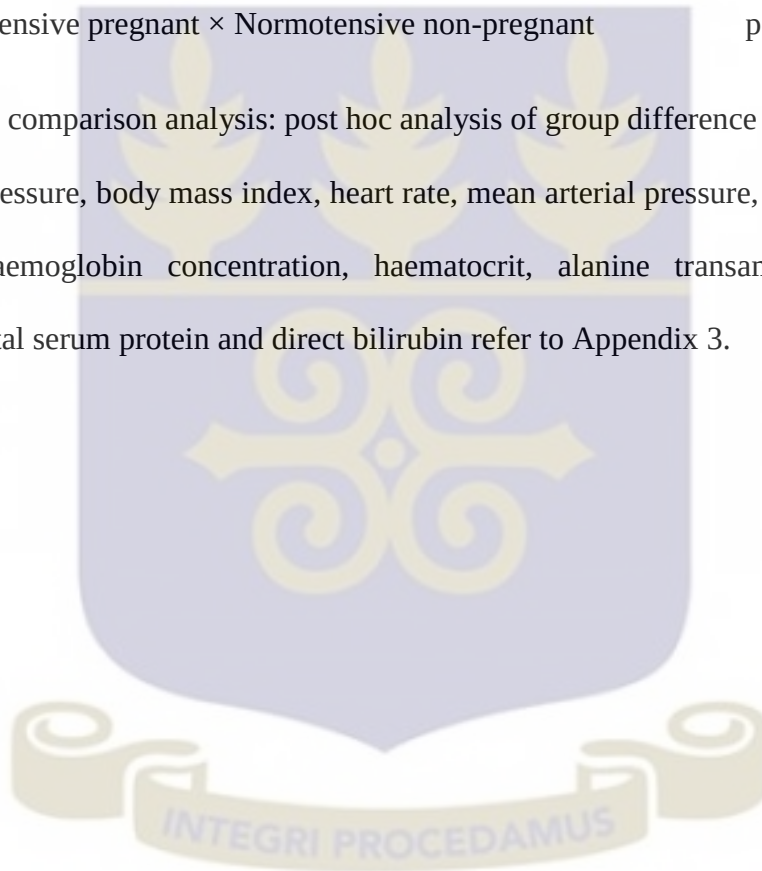
Pair wise comparison analysis: post hoc analysis of group differences were as follows:

Group differences that were not significant were excluded.

I. Diastolic Blood Pressure

- | | |
|--|-----------------|
| a. Preeclampsia × Normotensive pregnant | p-value = 0.000 |
| b. Preeclampsia × Normotensive non-pregnant | p-value = 0.000 |
| c. Normotensive pregnant × Normotensive non-pregnant | p-value = 0.001 |

For the pair wise comparison analysis: post hoc analysis of group difference for height, parity, systolic blood pressure, body mass index, heart rate, mean arterial pressure, serum creatinine, serum urea, haemoglobin concentration, haematocrit, alanine transaminase, aspartate transaminase, total serum protein and direct bilirubin refer to Appendix 3.



4.2 Comparing mean serum electrolyte and nitric oxide levels of preeclamptic, normotensive pregnant women and normotensive non-pregnant women

The mean serum magnesium, total calcium, ionised calcium, sodium and potassium levels in the three groups were analysed using a one-way analysis of variance (ANOVA) and a p-value <0.05 was adjudged significant. There was a statistically significant difference in serum magnesium levels, serum sodium levels, serum potassium levels and serum nitric oxide levels between preeclamptic, normotensive pregnant and normotensive non-pregnant women with p-values of 0.006, 0.009, 0.012 and 0.000 respectively (Table 4.2).

Table 4.2: Measured mean serum electrolytes and nitric oxide of preeclamptic, normotensive pregnant and normotensive non-pregnant women

PARAMETER	PREECLAMPTIC	NORMOTENSIVE PREGNANT	NORMOTENSIVE NON-PREGNANT	P-VALUE
	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	
n	30	100	32	
Serum Magnesium levels (mmol/l)	0.70 $\pm$ 0.15	0.77 $\pm$ 0.14	0.80 $\pm$ 0.10	0.006*
Total Serum Calcium levels (mmol/l)	2.13 $\pm$ 0.30	2.14 $\pm$ 0.34	2.20 $\pm$ 0.16	0.564
Serum Ionised Calcium levels (mmol/l)	1.15 $\pm$ 0.06	1.20 $\pm$ 0.11	1.19 $\pm$ 0.09	0.091
Serum Sodium Levels (mmol/l)	136.13 $\pm$ 4.17	139.90 $\pm$ 6.54	139.16 $\pm$ 4.64	0.009*
Serum Potassium levels (mmol/l)	3.45 $\pm$ 0.54	3.75 $\pm$ 0.47	3.61 $\pm$ 0.51	0.012*
Nitric Oxide levels (nM)	1178.78 $\pm$ 89.70	1308.30 $\pm$ 98.44	470.50 $\pm$ 78.21	0.000*

* Significant at $p \leq 0.05$; n – Sample size; Mean $\pm$ SD – Mean plus or minus standard deviation

Pair wise comparison analysis: post hoc analysis of group differences were as follows:

Group differences that were not significant were excluded.

I. Serum Magnesium

- | | |
|---|-----------------|
| a. Preeclampsia × Normotensive pregnant | p-value = 0.034 |
| b. Preeclampsia × Normotensive non-pregnant | p-value = 0.006 |

For the pair wise comparison analysis: post hoc analysis of group difference for serum sodium, serum potassium and serum nitric oxide, refer to Appendix 4.



4.3 Comparing mean serum electrolyte and nitric oxide levels of first, second and third trimester normotensive pregnant women

The mean serum magnesium, total calcium, ionised calcium, sodium and potassium levels in the three groups of normotensive pregnant women were analysed using a one-way analysis of variance (ANOVA) and a p-value <0.05 was adjudged significant. There was a statistically significant difference in serum sodium levels, serum ionised calcium levels and serum potassium levels between first, second and third trimester normotensive pregnant women with p-values of 0.000, 0.000, and 0.000 respectively (Table 4.3).

Table 4.3: Measured mean serum electrolytes and nitric oxide of first, second and third trimester normotensive pregnant women

PARAMETER	Trimester 1	Trimester 2	Trimester 3	P-value
	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	
n	33	37	30	
Serum Magnesium levels (mmol/l)	0.77 $\pm$ 0.11	0.77 $\pm$ 0.16	0.76 $\pm$ 0.14	0.950
Total Serum Calcium levels (mmol/l)	2.15 $\pm$ 0.16	2.13 $\pm$ 0.44	2.13 $\pm$ 0.35	0.967
Serum Sodium levels (mmol/l)	134.73 $\pm$ 5.11	142.68 $\pm$ 5.66	142.17 $\pm$ 5.66	0.000*
Serum Ionised Calcium levels (mmol/l)	1.14 $\pm$ 0.07	1.24 $\pm$ 0.13	1.21 $\pm$ 0.10	0.000*
Serum Potassium levels (mmol/l)	3.53 $\pm$ 0.52	3.76 $\pm$ 0.43	3.98 $\pm$ 0.36	0.000*
Nitric Oxide levels (nM)	1204.91 $\pm$ 290.95	1345.56 $\pm$ 80.24	1365.43 $\pm$ 95.46	0.783

* Significant at $p \leq 0.05$; n – Sample size; Mean $\pm$ SD – Mean plus or minus standard deviation

Pair wise comparison analysis: post hoc analysis of group differences were as follows:

Group differences that were not significant were excluded.

I. Serum Sodium of normotensive pregnant women

a. First trimester $\times$ Second trimester p-value = 0.000

b. First trimester $\times$ Third trimester p-value = 0.000

For the pair wise comparison analysis: post hoc analysis of group difference for serum calcium and serum ionised calcium, refer to Appendix 5.



4.4 Comparing mean arterial pressure of preeclamptic, normotensive pregnant women and normotensive non-pregnant women

There was a significant difference between the mean arterial pressure in preeclamptic, normotensive pregnant and normotensive non-pregnant women with $p=0.000$ on using one-way analysis of variance (ANOVA) test. Using the Post hoc analysis, there was a statistically significant difference between preeclamptic and normotensive pregnant women ($p=0.000$), preeclamptic and normotensive non-pregnant women ($p=0.000$) and normotensive pregnant women and normotensive non-pregnant women ($p=0.012$). The mean arterial pressure in preeclamptic, normotensive pregnant and normotensive non-pregnant women were 126.20 ± 20.86 , 82.33 ± 12.49 and 90.51 ± 9.71 mmHg respectively. A $p < 0.05$ was adjudged significant (Figure 4.1).

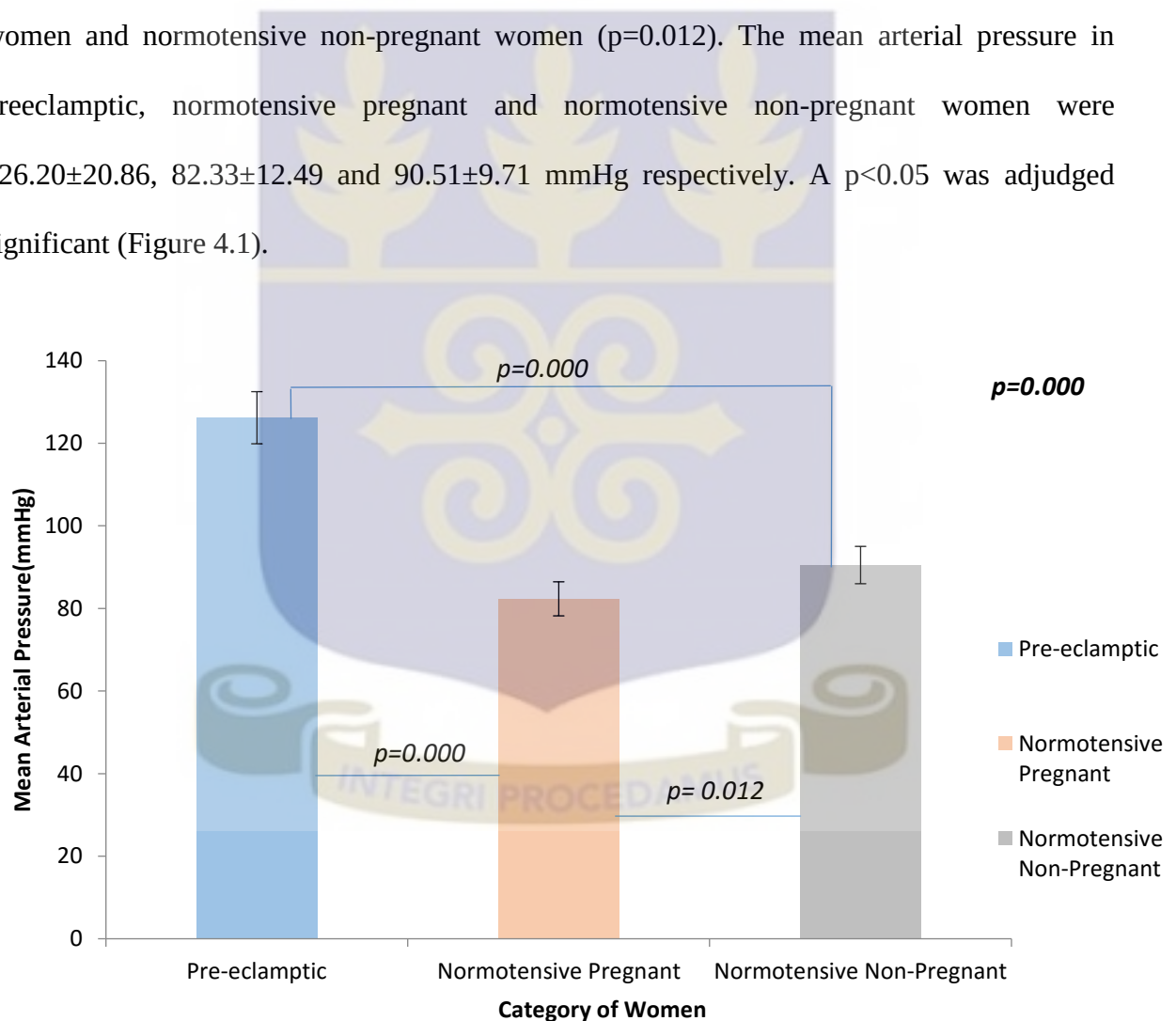


Figure 4.1: Mean arterial pressure (MAP) of preeclamptic, normotensive pregnant and normotensive non-pregnant women

4.5 Comparing mean heart rate of preeclamptic, normotensive pregnant women and normotensive non-pregnant women

There was a significant difference between the mean heart rate in preeclamptic, normotensive pregnant and normotensive non-pregnant women with $p=0.001$ on using one-way analysis of variance (ANOVA) test. Using the post hoc analysis, there was a statistically significant difference between preeclamptic and normotensive pregnant women ($p=0.001$), preeclamptic and normotensive non-pregnant women ($p=0.004$) but no statistically significant difference between normotensive pregnant women and normotensive non-pregnant women ($p=0.919$). The mean heart rate in preeclamptic, normotensive pregnant and normotensive non-pregnant women were 95.8 ± 13.24 , 85.71 ± 13.70 and 84.63 ± 13.81 bpm respectively. A $p < 0.05$ was adjudged significant (Figure 4.2).

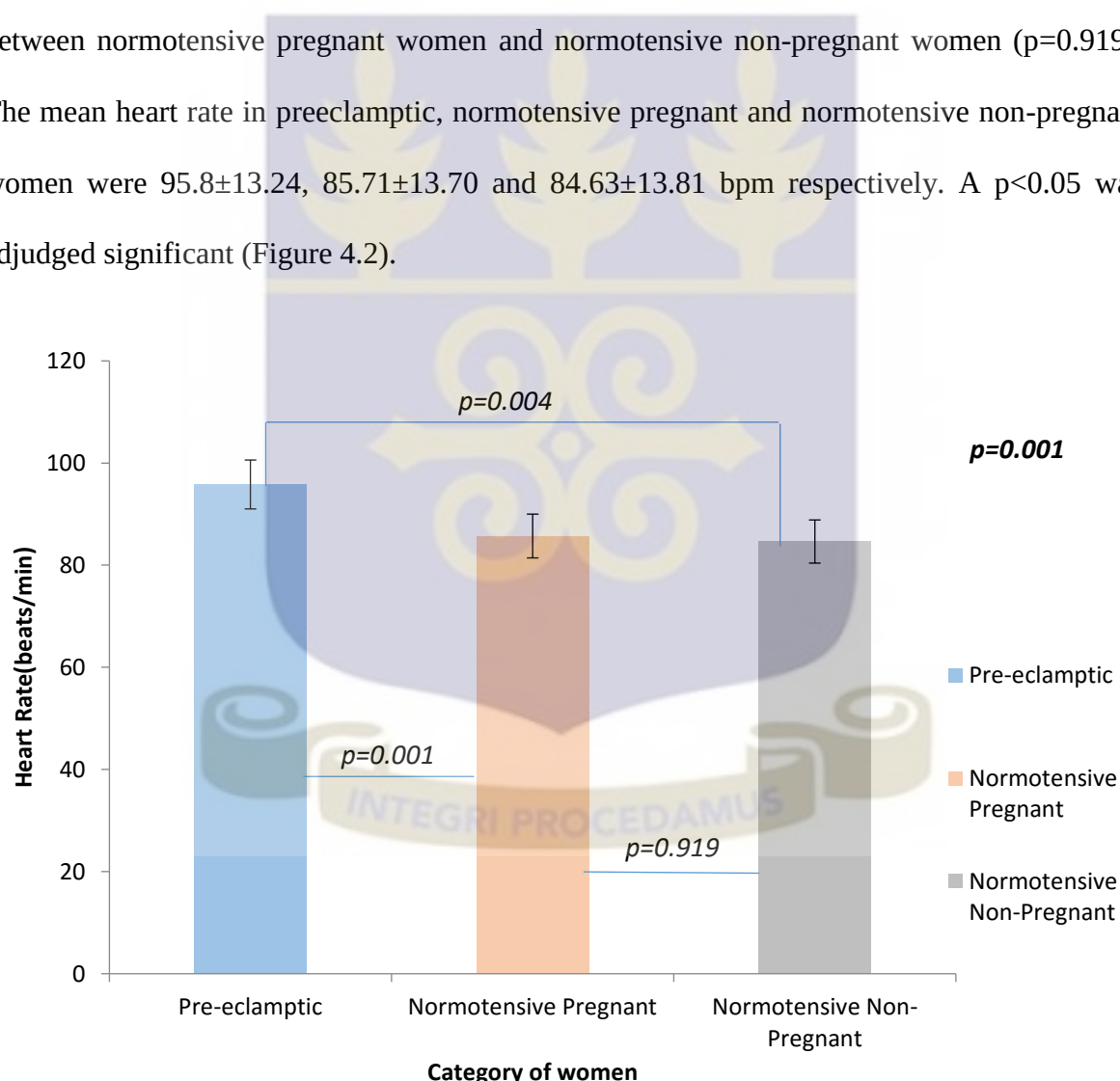


Figure 4.2: Mean heart rate in preeclamptic, normotensive pregnant and normotensive non-pregnant women

4.6 Comparing mean arterial pressure of first, second and third trimester normotensive pregnant women

There was no significant change in the mean arterial pressure of first, second and third trimester normotensive pregnant women ($p=0.725$). The mean arterial pressure of first, second and third trimester normotensive pregnant women were 81.82 ± 18.05 , 81.55 ± 8.55 and 83.87 ± 8.85 mmHg respectively. The mean arterial pressure of first, second and third trimester normotensive pregnant women were compared using one-way analysis of variance (ANOVA) and a p -value < 0.05 was adjudged to be significant (Figure 4.3).

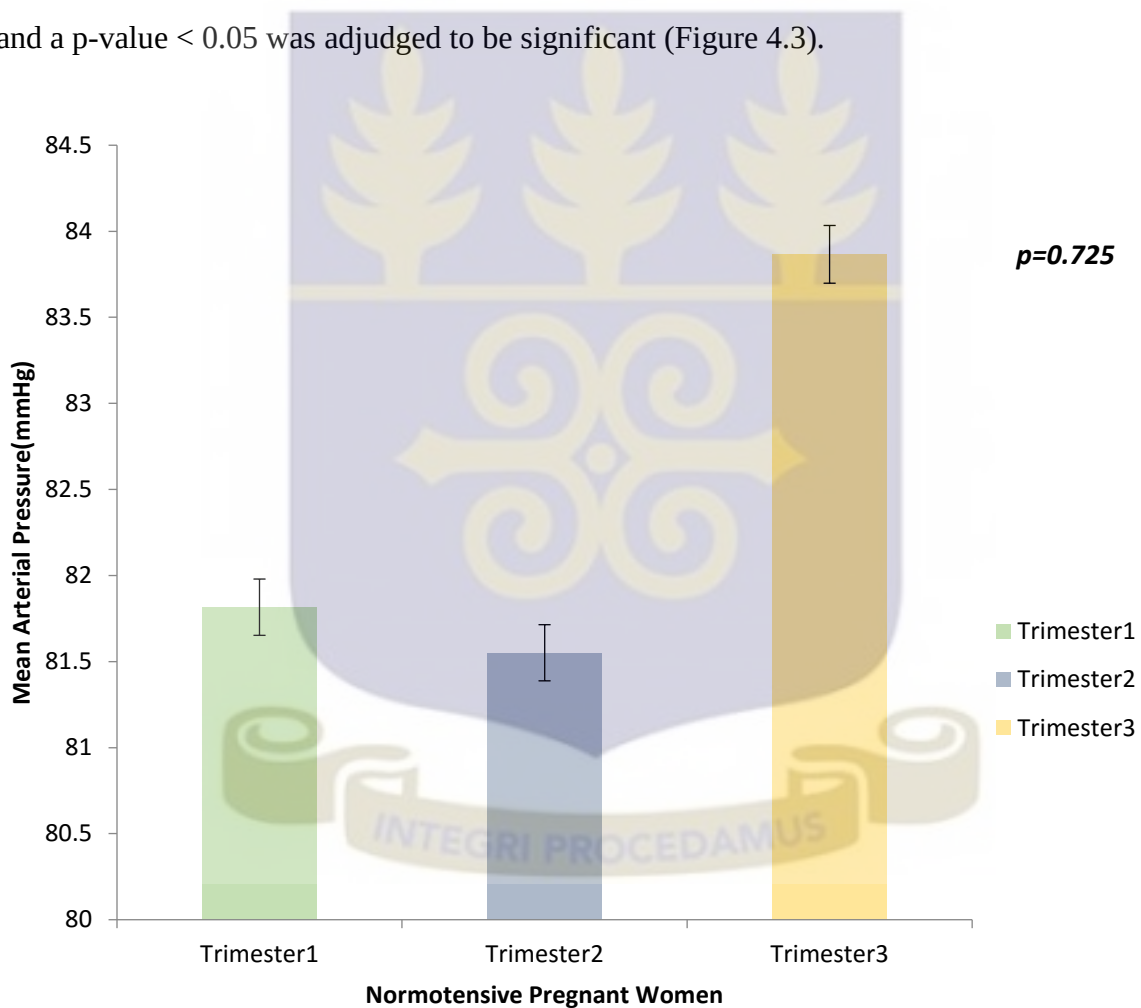


Figure 4.3: Mean arterial pressure (MAP) of first, second and third trimester normotensive pregnant women

4.7 Comparing mean heart rate of first, second and third trimester normotensive pregnant women

There was no significant change in the mean heart rate of first, second and third trimester normotensive pregnant women ($p=0.245$). The mean heart rate of first, second and third trimester normotensive pregnant women were 83.87 ± 14.27 , 84.51 ± 13.64 and 89.20 ± 12.91 bpm respectively. The mean heart rate of first, second and third trimester normotensive pregnant women were compared using one-way analysis of variance (ANOVA) and a p-value < 0.05 was adjudged to be significant (Figure 4.4).

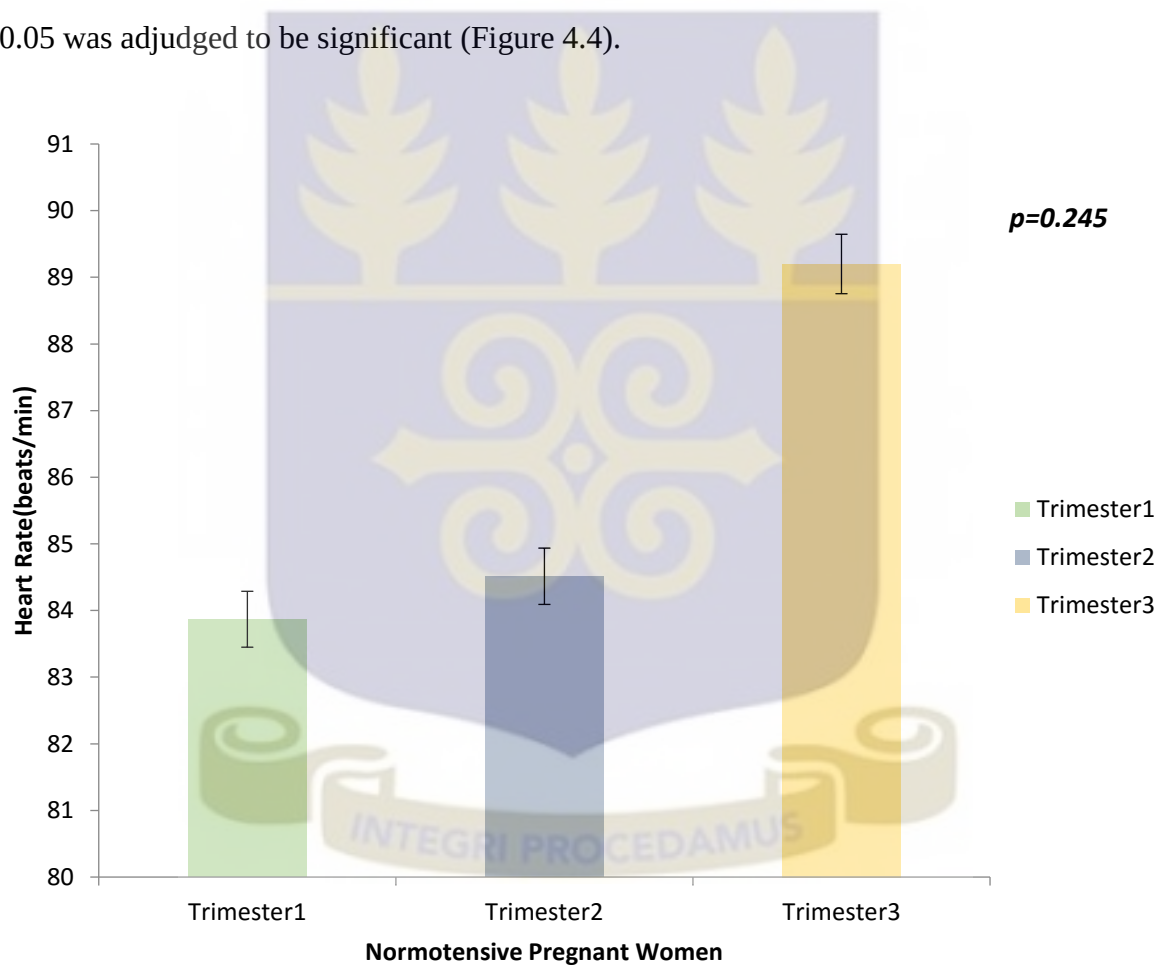


Figure 4.4: Mean heart rate (HR) of first, second and third trimester normotensive pregnant women

4.8 Comparing change in mean heart rate and mean arterial pressure of first, second and third trimester normotensive pregnant women

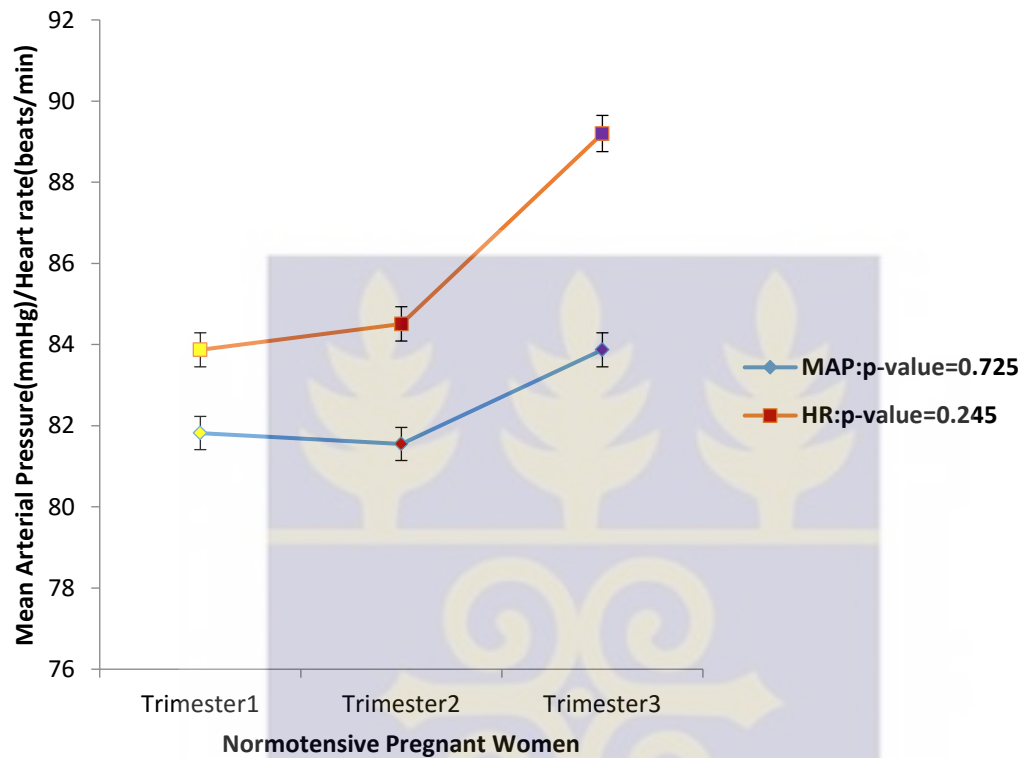


Figure 4.5: A line graph comparing change in mean heart rate (HR) and mean arterial pressure (MAP) of first, second and third trimester normotensive pregnant women

4.9 Comparing mean serum magnesium levels in preeclamptic, normotensive pregnant women and normotensive non-pregnant women

There was a significant difference between the serum magnesium levels in preeclamptic, normotensive pregnant and normotensive non-pregnant women ($p=0.006$) on using one-way analysis of variance (ANOVA) test. Using the Post hoc analysis, there was a statistically significant difference between preeclamptic and normotensive pregnant women ($p=0.034$), preeclamptic and normotensive non-pregnant women ($p=0.006$) but no statistically significant difference between normotensive pregnant women and normotensive non-pregnant women ($p=0.367$). The serum magnesium levels in preeclamptic, normotensive pregnant and normotensive non-pregnant women were 0.70 ± 0.15 , 0.77 ± 0.14 and 0.80 ± 0.10 mmol/L respectively. A $p<0.05$ was adjudged significant (Figure 4.6).

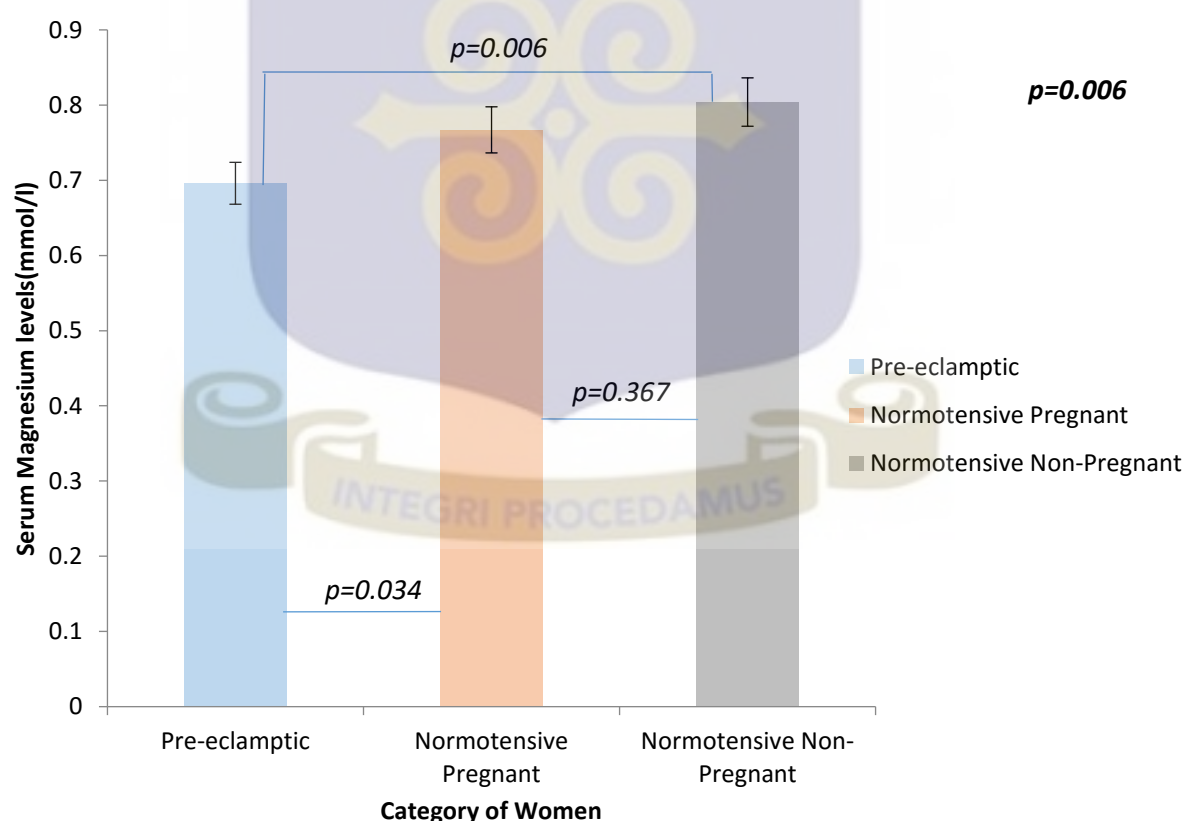


Figure 4.6: Mean serum magnesium levels in preeclamptic, normotensive pregnant and normotensive non-pregnant women

4.10 Comparing mean serum magnesium levels in first, second and third trimester normotensive pregnant women

There was no significant difference between the mean serum magnesium levels in first, second and third trimester normotensive pregnant women with $p=0.950$. The mean serum magnesium levels in first, second and third trimester normotensive pregnant women were 0.77 ± 0.11 , 0.77 ± 0.16 and 0.76 ± 0.14 mmol/L respectively. The mean serum magnesium levels in the three groups were compared using a one-way analysis of variance (ANOVA) and a $p<0.05$ was adjudged significant (Figure 4.7).

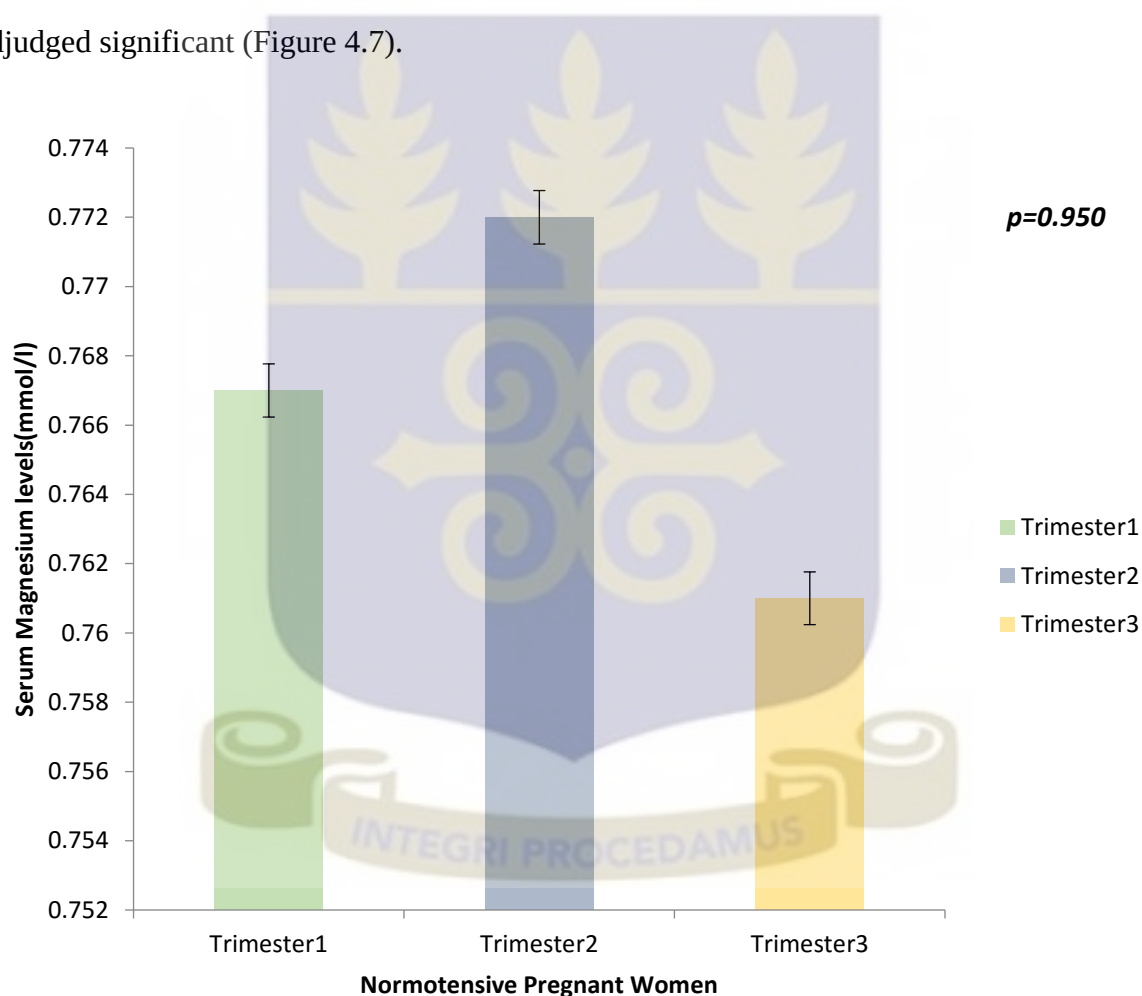


Figure 4.7: Mean serum magnesium levels in first, second and third trimester normotensive pregnant women

4.11 Comparing mean serum total calcium levels in preeclamptic, normotensive pregnant women and normotensive non-pregnant women

There was no significant difference between the mean serum total calcium levels in preeclamptic, normotensive pregnant and normotensive non-pregnant women with $p=0.564$. The mean serum total calcium levels in preeclamptic, normotensive pregnant and normotensive non-pregnant women were 2.13 ± 0.30 , 2.14 ± 0.34 and 2.20 ± 0.16 mmol/L respectively. The mean serum total calcium levels in the three groups were analysed using a one-way analysis of variance (ANOVA) and a $p<0.05$ was adjudged significant (Figure 4.8).

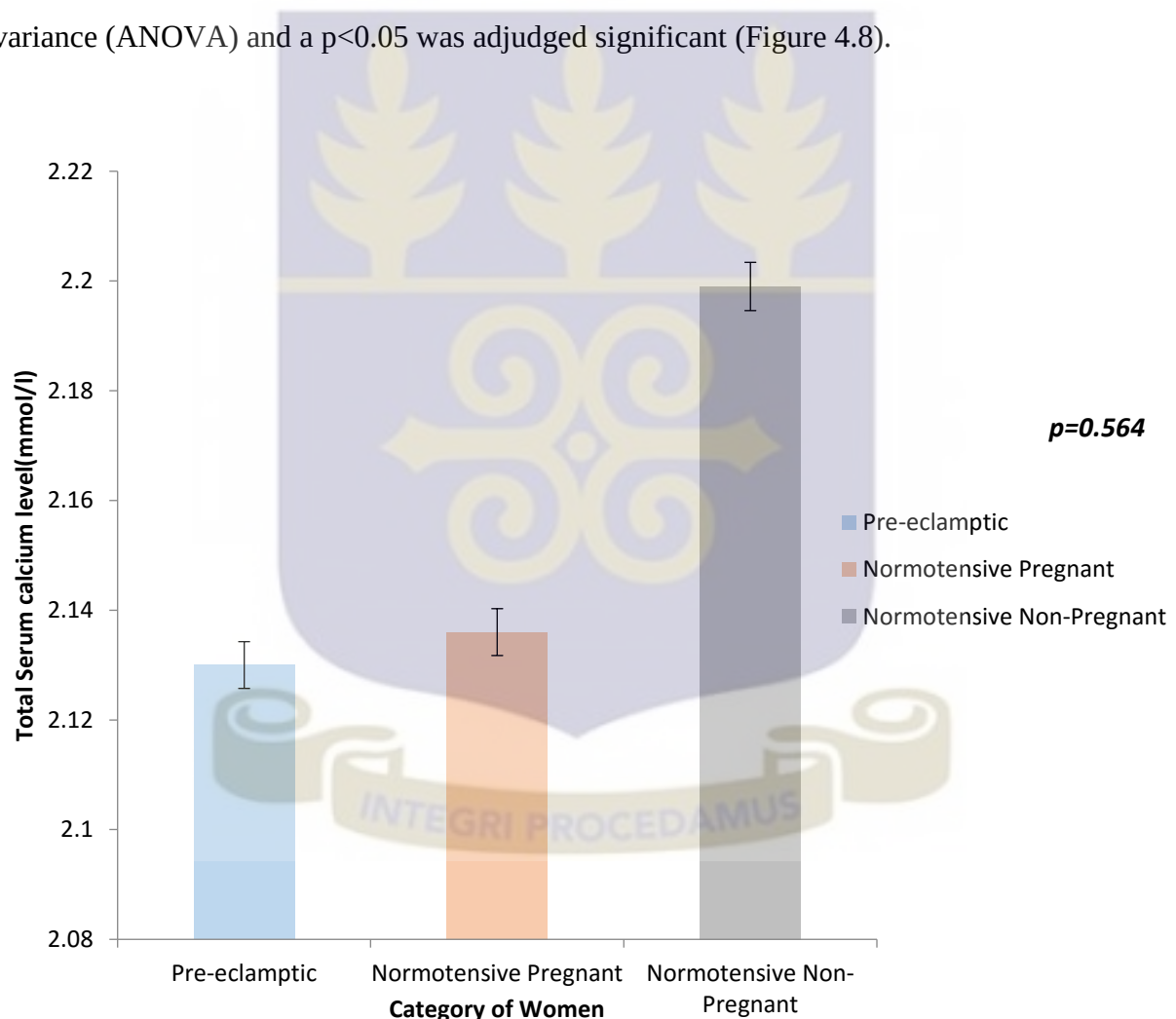


Figure 4.8: Mean serum total calcium levels in preeclamptic, normotensive pregnant and normotensive non-pregnant women

4.12 Comparing mean serum total calcium levels for first, second and third trimester normotensive pregnant women

There was no significant difference between the mean serum total calcium levels in first, second and third trimester normotensive pregnant women with $p=0.967$. The mean serum total calcium levels in first, second and third trimester normotensive pregnant women were 2.15 ± 0.16 , 2.13 ± 0.44 and 2.13 ± 0.35 mmol/L respectively. The mean serum total calcium levels in the three groups were compared using a one-way analysis of variance (ANOVA) and a $p<0.05$ was adjudged significant (Figure 4.9).

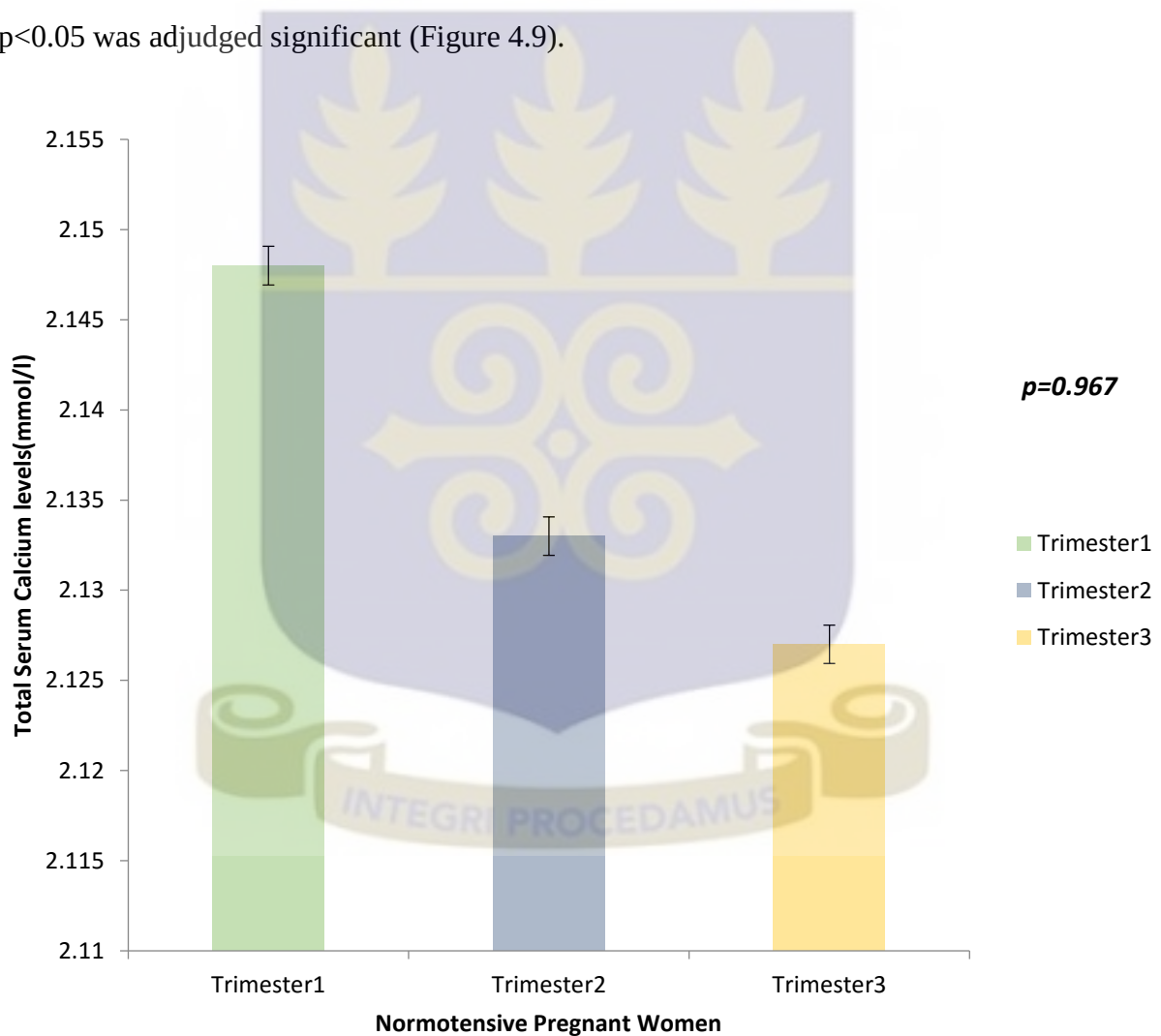


Figure 4.9: Mean serum total calcium levels for first, second and third trimester normotensive pregnant women

4.13 Comparing mean serum ionised calcium levels for preeclamptic, normotensive pregnant women and normotensive non-pregnant women

There was no significant difference between the mean serum ionised calcium levels in preeclamptic, normotensive pregnant and normotensive non-pregnant women with $p=0.091$. The mean serum total calcium levels in preeclamptic, normotensive pregnant and normotensive non-pregnant women were 1.15 ± 0.06 , 1.2 ± 0.11 and 1.19 ± 0.09 mmol/L respectively. The mean serum total calcium levels in the three groups were analysed using a one-way analysis of variance (ANOVA) and a $p<0.05$ was adjudged significant (Figure 4.10).

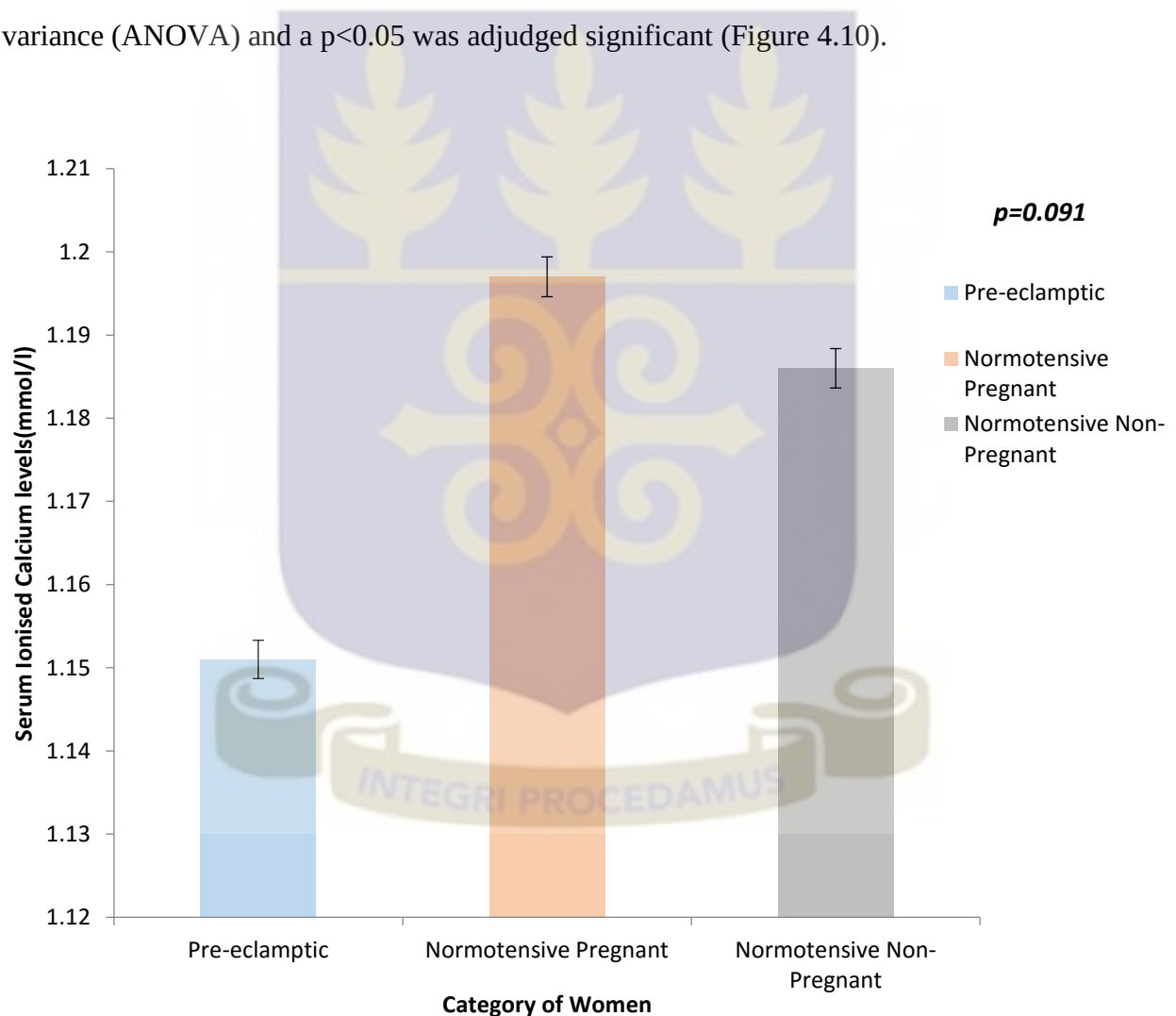


Figure 4.10: Mean serum ionised calcium levels for preeclamptic, normotensive pregnant and normotensive non-pregnant women

4.14 Comparing mean serum ionised calcium levels for first, second and third trimester normotensive pregnant women

There was a significant difference between the serum ionised calcium levels in first, second and third trimester normotensive pregnant women ($p=0.000$) on using one-way analysis of variance (ANOVA) test. Using the Post hoc analysis, there was a statistically significant difference between first and second trimester normotensive pregnant women ($p=0.000$), first and third trimester normotensive pregnant women ($p=0.023$) but no statistically significant difference between second and third trimester normotensive pregnant women ($p=0.334$). The serum ionised calcium levels in first, second and third trimester normotensive pregnant women were 1.14 ± 0.07 , 1.24 ± 0.13 and 1.21 ± 0.10 mmol/L respectively. A $p<0.05$ was adjudged significant (Figure 4.11).

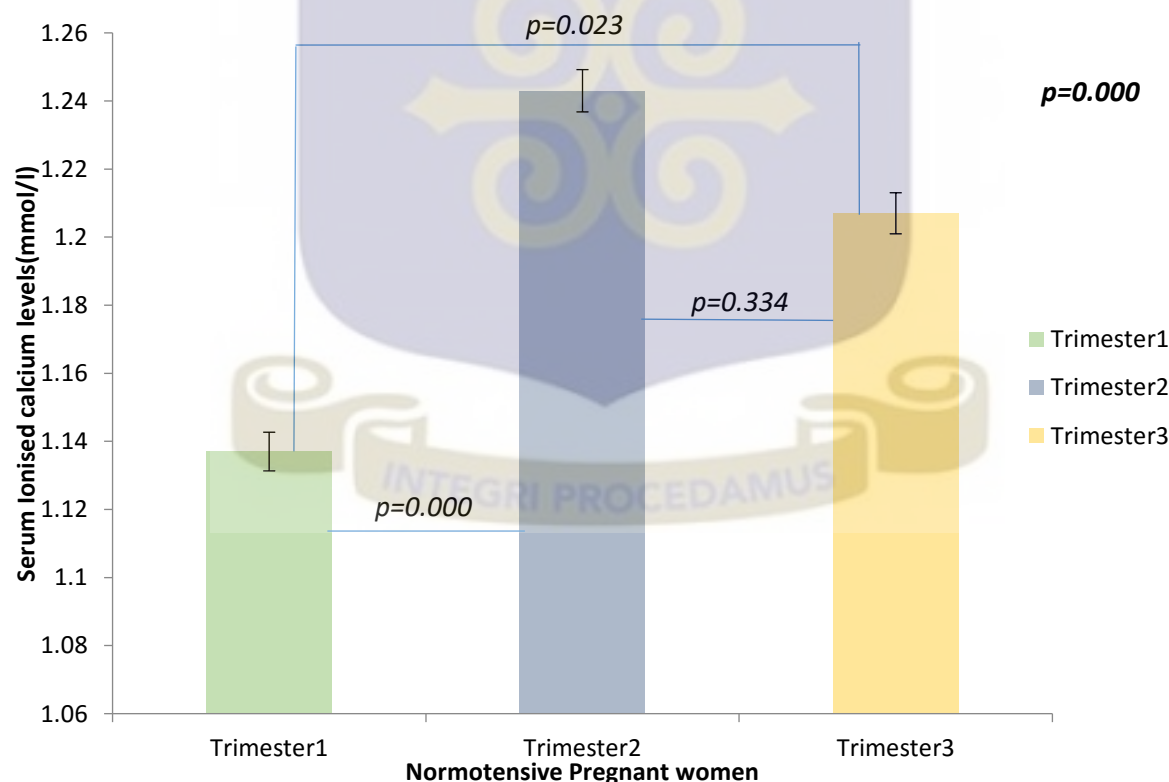


Figure 4.11: Mean serum ionised calcium levels for first, second and third trimester normotensive pregnant women

4.15 Comparing mean serum sodium levels for preeclamptic, normotensive pregnant women and normotensive non-pregnant women

There was a significant difference between the serum sodium levels in preeclamptic, normotensive pregnant and normotensive non-pregnant women ($p=0.009$) on using one-way analysis of variance (ANOVA) test. Using the Post hoc analysis, there was a statistically significant difference between preeclamptic and normotensive pregnant women ($p=0.006$) but no statistically significant difference between preeclamptic and normotensive non-pregnant women ($p=0.106$) and normotensive pregnant women and normotensive non-pregnant women ($p=0.805$). The serum sodium levels in preeclamptic, normotensive pregnant and normotensive non-pregnant women were 136.13 ± 4.17 , 139.00 ± 6.54 and 139.16 ± 4.64 mmol/L respectively. A $p<0.05$ was adjudged significant (Figure 4.12).

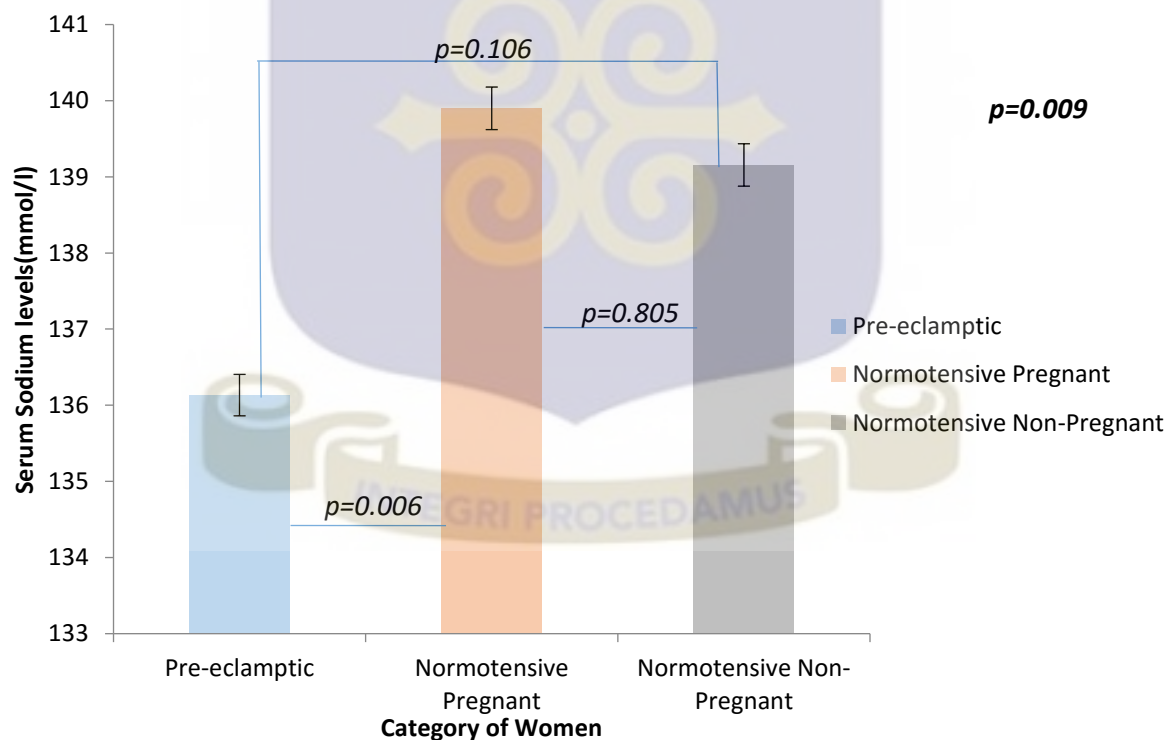


Figure 4.12: Mean serum sodium levels for preeclamptic, normotensive pregnant and normotensive non-pregnant women

4.16 Comparing mean serum sodium levels for first, second and third trimester normotensive pregnant women

There was a significant difference between the serum sodium levels in first, second and third trimester normotensive pregnant women ($p=0.000$) on using one-way analysis of variance (ANOVA) test. Using the Post hoc analysis, there was a statistically significant difference between first and second trimester normotensive pregnant women ($p=0.000$), first and third trimester normotensive pregnant women ($p=0.000$) but no statistically significant difference between second and third trimester normotensive pregnant women ($p=0.924$). The serum sodium levels first, second and third trimester normotensive pregnant women were 134.73 ± 5.11 , 142.68 ± 5.66 and 142.17 ± 5.66 mmol/L respectively. A $p < 0.05$ was adjudged significant (Figure 4.13).

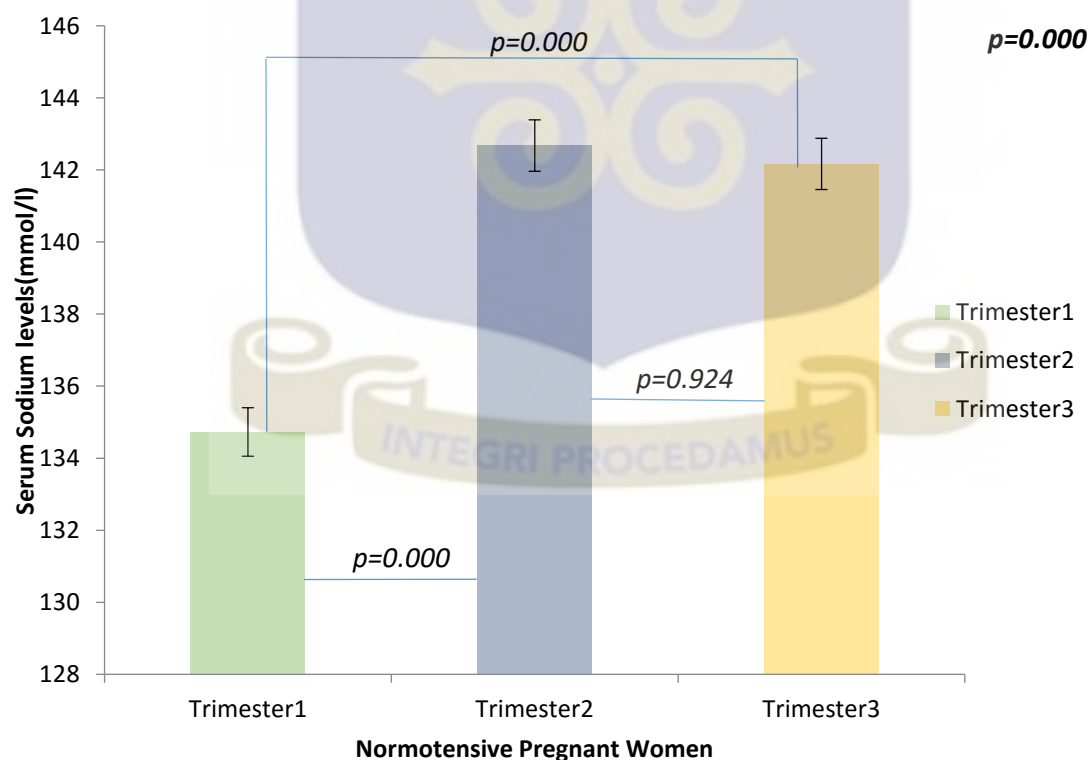


Figure 4.13: Mean serum sodium levels for first, second and third trimester normotensive pregnant women

4.17 Comparing mean serum potassium levels for preeclamptic, normotensive pregnant women and normotensive non-pregnant women

There was a significant difference between the serum potassium levels in preeclamptic, normotensive pregnant and normotensive non-pregnant women ($p=0.012$) on using one-way analysis of variance (ANOVA) test. Using the Post hoc analysis, there was a statistically significant difference between preeclamptic and normotensive pregnant women ($p=0.011$) but no statistically significant difference between preeclamptic and normotensive non-pregnant women ($p=0.426$) and normotensive pregnant women and normotensive non-pregnant women ($p=0.324$). The serum potassium levels in preeclamptic, normotensive pregnant and normotensive non-pregnant women were 3.45 ± 0.54 , 3.75 ± 0.47 and 3.61 ± 0.51 mmol/L respectively. A $p<0.05$ was adjudged significant (Figure 4.14).

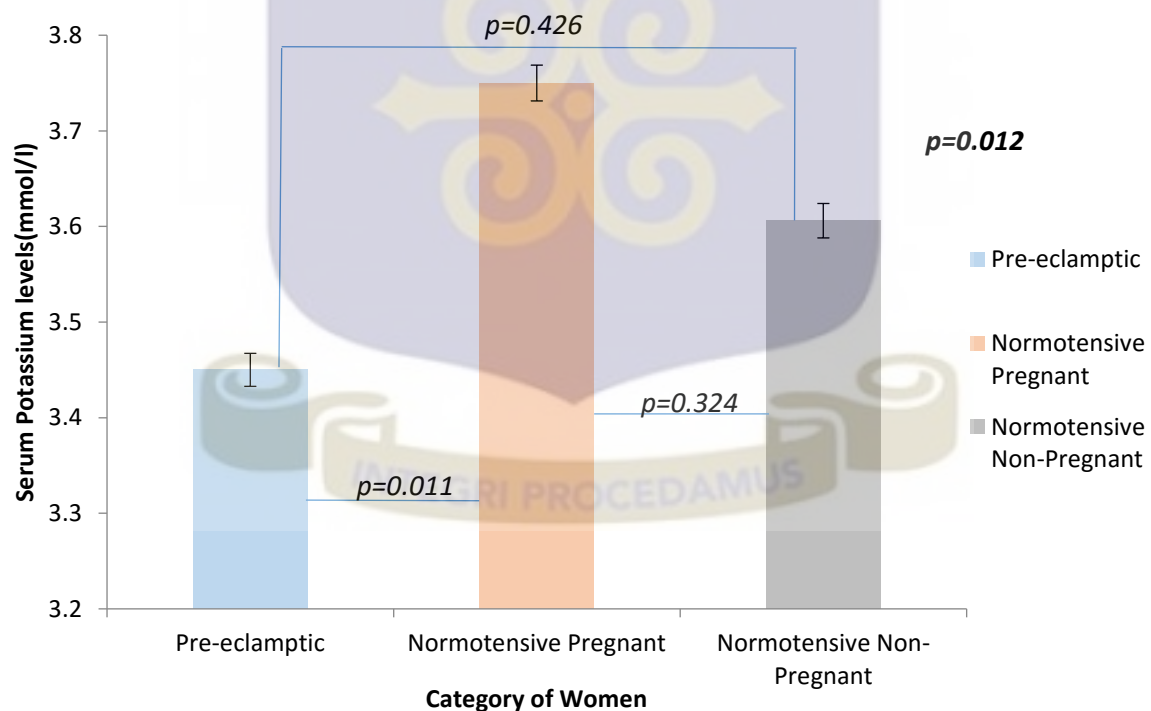


Figure 4.14: Mean serum potassium levels for preeclamptic, normotensive pregnant and normotensive non-pregnant women

4.18 Comparing mean serum potassium levels for first, second and third trimester normotensive pregnant women

There was a significant difference between the serum potassium levels in first, second and third trimester normotensive pregnant women ($p=0.000$) on using one-way analysis of variance (ANOVA) test. Using the Post hoc analysis, there was a statistically significant difference between first and third trimester normotensive pregnant women ($p=0.000$) but no statistically significant difference between first and second trimester normotensive pregnant women ($p=0.088$) and second and third trimester normotensive pregnant women ($p=0.099$). The serum potassium levels in first, second and third trimester normotensive pregnant women were 3.53 ± 0.52 , 3.76 ± 0.43 and 3.98 ± 0.36 mmol/L respectively. A $p<0.05$ was adjudged significant (Figure 4.15).

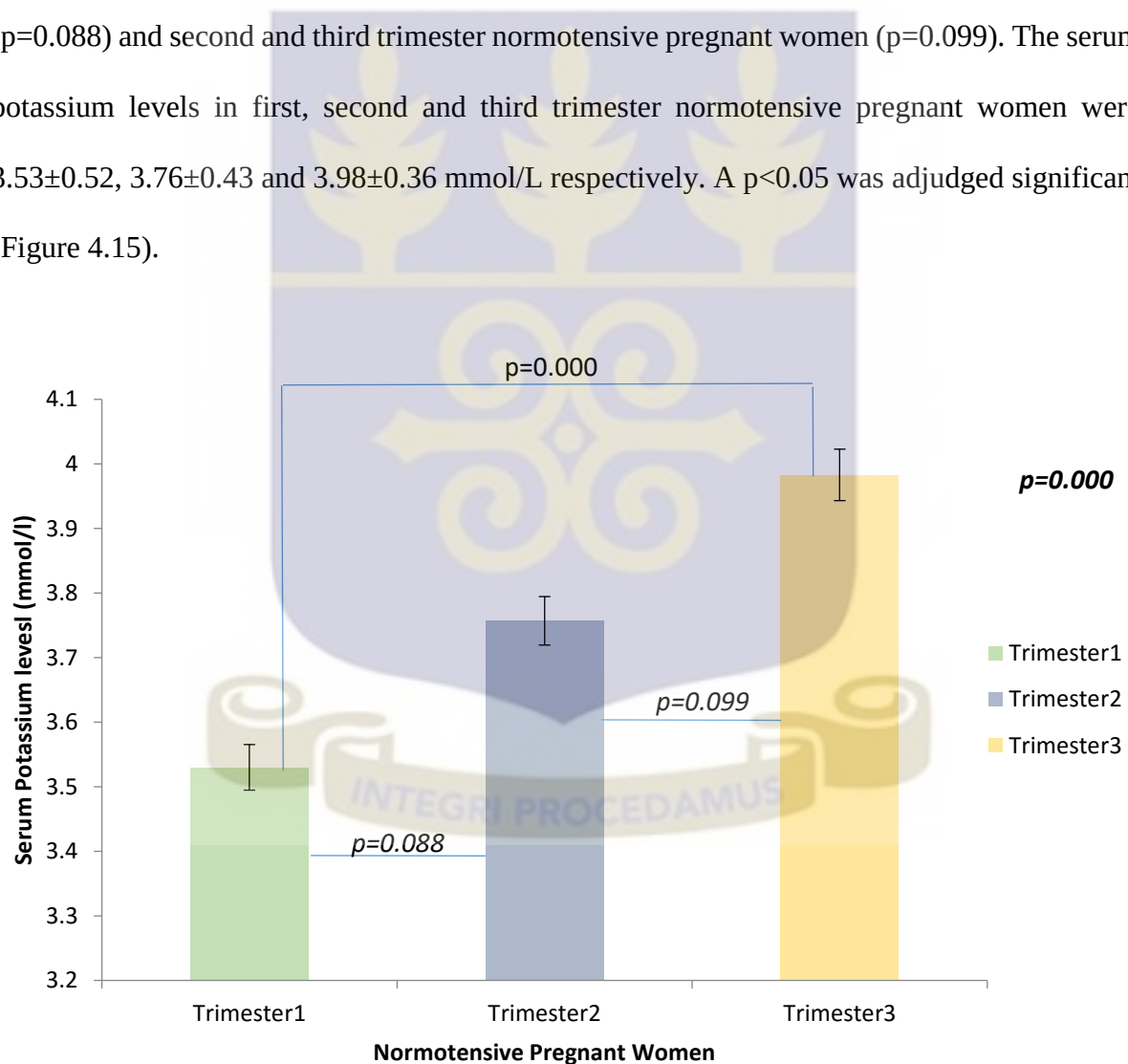


Figure 4.15: Mean serum potassium levels for first, second and third trimester normotensive pregnant women

4.19 Comparing mean serum nitric oxide levels for preeclamptic, normotensive pregnant women and normotensive non-pregnant women

There was a significant difference between the serum nitric oxide levels in preeclamptic, normotensive pregnant and normotensive non-pregnant women ($p=0.000$) on using one-way analysis of variance (ANOVA) test. Using the Post hoc analysis, there was a statistically significant difference between preeclamptic and normotensive non-pregnant women ($p=0.001$), normotensive pregnant and normotensive non-pregnant women ($p=0.000$) but no statistically significant difference between preeclamptic and normotensive pregnant women ($p=0.447$). The serum nitric oxide levels in preeclamptic, normotensive pregnant and normotensive non-pregnant women were 1178.78 ± 89.70 , 1308.30 ± 98.44 and 470.50 ± 78.21 nM respectively. A $p < 0.05$ was adjudged significant (Figure 4.16).

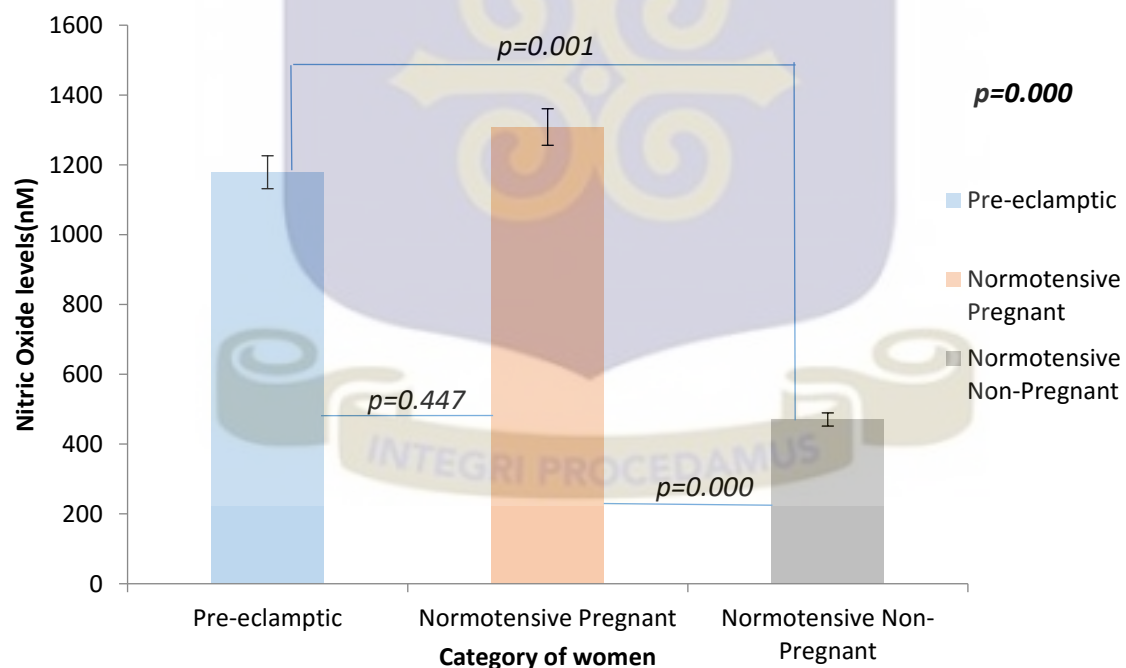


Figure 4.16: Mean serum nitric oxide levels for preeclamptic, normotensive pregnant and normotensive non-pregnant women

4.20 Comparing mean serum nitric oxide levels for first, second and third trimester normotensive pregnant women

There was no significant difference between the mean serum nitric oxide levels in first, second and third trimester normotensive pregnant women ($p=0.783$). The mean serum nitric oxide in first, second and third trimester normotensive pregnant women were 1204.91 ± 290.95 , 1345.56 ± 80.24 and 1365.43 ± 95.46 nM respectively. The mean serum total calcium levels in the three groups were analysed using a one-way analysis of variance (ANOVA) and a $p < 0.05$ was adjudged significant (Figure 4.17).

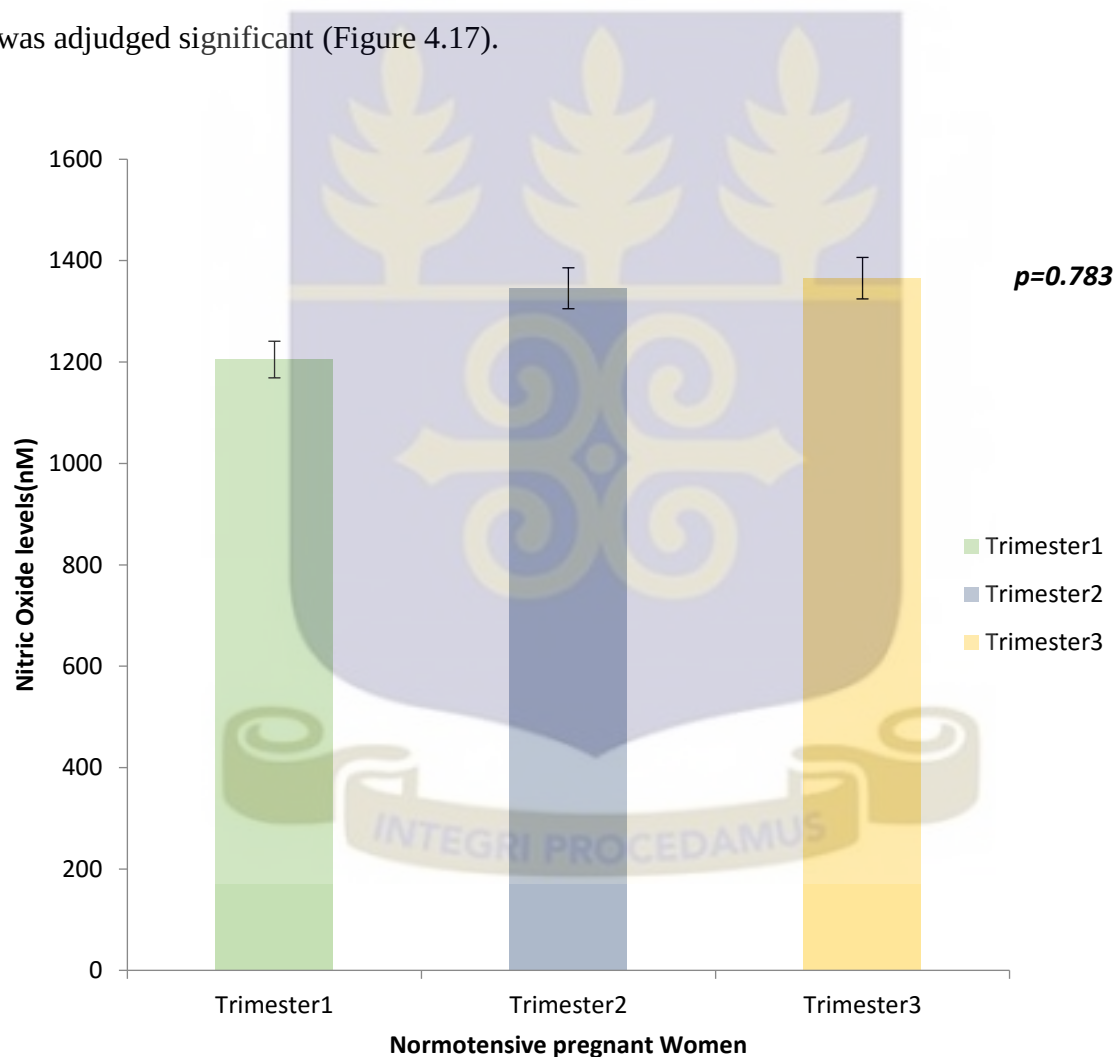


Figure 4.17: Mean serum nitric oxide levels for first, second and third trimester normotensive pregnant women

CHAPTER FIVE

5.0 DISCUSSION

5.1 Introduction

Preeclampsia is a multisystem disorder of widespread vascular endothelial malfunction and vasospasm occurring after 20 weeks of gestation, and presenting clinically as hypertension and proteinuria with or without pathological oedema (Sibai, 2003; Tavana & Hosseinmirzaei, 2013).

The aetiology of preeclampsia has been postulated to lie in the placenta though it remains unknown (Smith & Kenny, 2006; Uzan *et al.*, 2011). However, abnormalities in serum major cations have been said to play a significant role in the pathophysiology of preeclampsia. In recent times more emphasis has been laid on the relationship between maternal serum levels of mineral elements and occurrence of preeclampsia (Adam *et al.*, 2001; Cunningham *et al.*, 2005).

Recent epidemiological studies shows that mortality rate from preeclampsia is highest in low and middle income countries (Khan *et al.*, 2006). Preeclampsia is also associated with an increase in perinatal mortality, increase in congenital neurological disability and premature birth (Goldenberg & Rouse, 1998; Lain & Roberts, 2002; McDonald *et al.*, 2008).

The main objective for this study is to determine the role of major serum cations (magnesium, calcium, sodium and potassium) in the pathophysiology of preeclampsia by measuring and comparing maternal serum levels of these major serum cations with preeclamptic, normotensive pregnant and normotensive non-pregnant women.

5.2 Changes in mean arterial pressure (MAP) and heart rate (HR) during normal pregnancy and preeclampsia

From this study, the mean arterial blood pressures obtained were significantly different among the preeclamptics in comparison to the normotensive pregnant and the normotensive non-pregnant women as shown in Figure 4.1. From this result the mean arterial blood pressures drops significantly during normal pregnancy as compared to normotensive non-pregnant state and rises significantly when there is a development of preeclampsia. The above finding is consistent with that of Grindheim and colleagues (Grindheim *et al.*, 2012)

Also this study reveals a significant increase in the heart rate of preeclamptic women as compared to normotensive pregnant and normotensive non-pregnant women as shown in Figure 4.2.

From this result the mean heart rate during normal pregnancy rises as compared to the normotensive non-pregnant state but the difference was not significant. This finding agrees with those of previous authors (Carruth *et al.*, 1981; Clapp & Capeless, 1997; Grindheim *et al.*, 2012; Mahendru *et al.*, 2014).

During normal pregnancy, there are profound cardiovascular changes that leads to a drop in arterial blood pressure. This drop in blood pressure is mediated by peripheral vasodilatation, nitric oxide synthesis, upregulation of oestradiol and production of prostacyclin (PGI₂). There is approximately 30% reduction in systemic vascular resistance as a result of peripheral vasodilatation. To compensate for the drop in mean arterial pressure during normal pregnancy, there is an increase in cardiac output via an increase in heart rate and stroke volume. Though stroke volume reduces towards term, the increased heart rate is maintained so as to preserve the cardiac output (Mahendru *et al.*, 2014; Soma-Pillay *et al.*, 2016).

From Figure 4.3 and Figure 4.5, it is evident that mean arterial pressure drops from the beginning of pregnancy and nadirs at the second trimester and thereafter progressively increases till term. This change noticed in this study though not significant, is confirmed in literature (Grindheim *et al.*, 2012; Sanghavi & Rutherford, 2014). Majority of the reduction in blood pressure occurs very early in pregnancy (around 6 weeks of gestation) compared with preconception values (Mahendru *et al.*, 2014). Because these changes happen early in pregnancy it is important to compare blood pressure measurements with preconception values (Figure 4.1) instead of early pregnancy values when fluctuations might have already occurred (Sanghavi & Rutherford, 2014). This may explain the statistically non-significance of the change noticed among normotensive pregnant women.

Unlike blood pressure which drops in second trimester during normal pregnancy, heart rate progressively increases throughout normal pregnancy reaching a maximum in the third trimester (Figure 4.4 and Figure 4.5). This increase in heart rate is to compensate for the maintenance of a normal cardiac output on account of a drop in blood pressure during pregnancy (Soma-Pillay *et al.*, 2016). However, from this study, even though there was a progressive increase in heart rate from first to third trimester, this change was not significant.

5.3 Serum Magnesium Levels and Preeclampsia

Serum magnesium levels was found to be significantly lower in preeclamptic women compared to normotensive pregnant women and normotensive non-pregnant women as shown in Figure 4.6. However, there was no statistically significant difference between the serum levels of magnesium in normotensive pregnant women and normotensive non-pregnant women as shown in Figure 4.6. The results obtained in this study is consistent with previous studies that have demonstrated significant reduction in serum levels of magnesium in preeclampsia (Akinloye *et al.*, 2013; Catov *et al.*, 2009; Jain *et al.*, 2010; Nygaard *et al.*, 2008; Roberts *et al.*, 2010).

The marked reduction in serum levels of magnesium seen in this study in preeclampsics and other previous studies is attributed to the role of magnesium in vascular smooth muscle relaxation (Sukonpan & Phupong, 2005), vascular endothelial dysfunction (Haenni *et al.*, 2002; Shechter *et al.*, 2000) and mediation of production of inflammatory markers (Chacko *et al.*, 2010).

Magnesium is the second most abundant intracellular cation and it is involved in various biochemical processes (Barbagallo *et al.*, 2010). Magnesium affects blood pressure by modulating vascular tone. Variations in extracellular magnesium alters the production and release of nitric oxide (NO), causing an alteration of arterial smooth muscle tone by affecting calcium concentration (Sanjuliani, de Abreu Fagundes, & Francischetti, 1996). Increase in extracellular magnesium inhibits calcium influx and vice versa. This effect is via calcium channels. Decreased intracellular concentrations of magnesium activates inositol triphosphate (IP3) mediated mobilization of intracellular calcium and therefore decreases Ca^{2+} -ATPase activity. This consequently decreases calcium efflux and uptake by the sarcoplasmic system thereby causing accumulation and increase in calcium in the cytosol which crucially affects vasoconstriction. Raised levels of intracellular concentrations of magnesium results in a reduction in cytosolic free calcium thereby causing vasodilatation (Griendling *et al.*, 1986). Therefore, magnesium acts as a blocker of calcium channels and thereby decreases the release of calcium and hence a decrease in vascular resistance. Magnesium also stimulates Na^{+} - K^{+} -ATPase pump and therefore controls the balance of electrolytes that is involved in the maintenance of homeostasis (Sontia & Touyz, 2007).

Low serum magnesium levels also affect the renin-angiotensin-aldosterone system and therefore the development of preeclampsia. High plasma renin activity has been noted in patients with low serum magnesium levels (Resnick *et al.*, 1983).

Low serum magnesium levels is also known to cause hypertrophic vascular remodelling therefore altering the mechanical properties of the vascular system and hence possible development of preeclampsia (Laurant *et al.*, 2000).

Low serum magnesium levels is also associated with a release of pro-inflammatory and oxidative stress markers and cell growth properties modulation. Magnesium has an antioxidant property which may prevent the deadly effects of above mediators on the vasculature and therefore preventing a vasoconstriction (Laurant & Touyz, 2000).

Endothelial dysfunction noted in preeclampsia is also associated with low serum magnesium levels. Endothelial dysfunction causes an imbalance in endothelial production of mediators that regulate vascular tone, fibrinolysis, coagulation and platelet aggregation. This endothelial dysfunction in preeclampsia is believed to be associated with a reduction in nitric oxide levels as well as alteration in other endothelial derived vasoactive substances mainly prostacyclin, angiotensin II and endothelin I (Krause *et al.*, 2011; Meher & Duley, 2007; Moncada *et al.*, 1991; Northcott & Watts, 2004). However, there are controversial views in literature concerning the levels of nitric oxide in preeclamptics (Diejomaoh *et al.*, 2004).

The mean serum magnesium levels among the first, second and third trimester normotensive pregnant women measured were not significantly different in this study as shown in Table 4.3. Furthermore, there was no statistical difference in the serum magnesium levels within the normotensive pregnant patients as per their gestation as shown in Figure 4.7. This result is consistent with previous studies that have also demonstrated no significant reduction in the levels of serum magnesium in normotensive pregnant women (Baloch, Shaikh, & Jaffary, 2012; Golmohammad *et al.*, 2008; Kumru *et al.*, 2003; Punthumapol & Kittichotpanich, 2008; Zeni *et al.*, 2003). However, in a study done in India (Pathak, Kapoor, Kapil, & Dwivedi, 2003), the mean serum levels of magnesium in the normotensive pregnant women were slightly lower

than found in this study whilst the mean serum magnesium levels reported among normal pregnant women in Thailand (Punthumapol & Kittichotpanich, 2008) were similar to the finding of this study.

It has been noted that in normal pregnancy serum magnesium levels may slightly reduce as the gestation progresses. This slight reduction is usually seen in the latter part of the gestation and it is attributed to haemodilution (Chesley, 1972; Pathak *et al.*, 2003; Sheldon, Aspillaga, Smith, & Lind, 1985), renal clearance during pregnancy and consumption of magnesium by the growing foetus (Williams & Galerneau, 2002) as shown in Figure 4.7. However, this reduction has been found not to be significant (Baloch *et al.*, 2012)

5.4 Serum Calcium Levels and Preeclampsia

Serum levels of both total and ionised calcium was not statistically significant between preeclamptic, normotensive pregnant and normotensive non-pregnant women as shown in Figure 4.8 and Figure 4.10. Calcium plays an important role in smooth muscle contraction and regulation of water balance in cells. The effect of calcium on blood pressure is explained by the action of calcitrophic hormones on intracellular calcium. Calcium influx in various cells including vascular smooth muscle is stimulated by 1, 25 -dihydroxycholecalciferol. This repressor effect is rapid and promotes vascular muscle contraction and therefore increase in vascular resistance. Increase in intracellular calcium serves as a second messenger in excitation-contraction coupling for vascular smooth muscle (Jain *et al.*, 2010). Hence, reduced calcium intake and therefore reduced serum calcium which may stimulate 1, 25 – dihydroxycholecalciferol is expected to increase peripheral vascular resistance and vice versa. Therefore, alterations in plasma calcium concentration can lead to alterations in blood pressure. Increase in cellular calcium concentration with a fall in serum calcium levels can therefore explain the vasoconstrictive effects seen in preeclamptic mothers (Sukonpan & Phupong,

2005). Dietary deficiency of calcium during pregnancy has been implicated in preeclampsia (Jain *et al.*, 2010).

In this study, the serum levels of both total and ionised calcium was not statistically significant between preeclamptic, normotensive pregnant and normotensive non-pregnant women as shown in both Figure 4.8 and Figure 4.10. This finding is consistent with some results in literature (Bera *et al.*, 2011; Golmohammad *et al.*, 2008; Salari & Eftekhari, 2005; Vahidrodsari *et al.*, 2008).

The above finding may be explained following the observation that preeclamptic women have a reduced level of daily urinary calcium excretion and fractional excretion of calcium in comparison to normotensive pregnant women. Researches in preeclamptic women have demonstrated significantly reduced 1,25-dihydroxycholecalciferol compared with normotensive pregnant women (Hojo & August, 1997). However, this finding has not been consistently proven. Other researchers have also noticed a raised level of parathyroid hormone (PTH) in preeclamptic women (Jain *et al.*, 2010; Punthumapol & Kittichotpanich, 2008; Sukonpan & Phupong, 2005), and again this has not been consistently proven. Therefore, it can be explained that preeclamptic women may have normal serum calcium levels as a consequence of a decrease in 1, 25-dihydroxycholecalciferol or dietary calcium causing a reduced intestinal absorption of calcium with resultant decrease in serum ionised calcium. As a consequence of this PTH increases as a normal response to the low serum ionised calcium or as a result of lower levels of 1, 25-dihydroxycholecalciferol. The raised PTH level then causes increased reabsorption of calcium in the intestine and the distal tubule causing hypocalciuria (Sanchez-Ramos, Sandroni, Andres, & Kaunitz, 1991; Suarez, Trelles, & Miyahira, 1996; Taufield *et al.*, 1987), which may normalise the serum calcium levels in preeclamptic women. Parathyroid hormone secretion is also associated with release of renin from the kidneys which

can cause vasoconstriction and retention of sodium and water and therefore preeclampsia (Hacker, Fung, & King, 2012).

The finding of no significant difference in serum calcium levels between preeclamptics and normotensive pregnant women as compared to other studies can also be attributed to the differences in dietary habits and differences in genetic pools of our population as compared to those of other studies. However, the dietary history of the participants in this study was not elucidated.

However, the finding of no significant difference in serum calcium levels of preeclamptics and normotensive pregnant women contradicts the findings of other studies where a significant reduction in serum levels of calcium was noted in preeclamptic and pregnancy induced hypertensive women as compared to normotensive pregnant women (Akhtar *et al.*, 2011; Indumati *et al.*, 2011; Kumru *et al.*, 2003; Mohieldein *et al.*, 2007; Punthumapol & Kittichotpanich, 2008). This is supported by various studies which identified calcium supplementation as reducing the risk of developing preeclampsia (Arevalo-Herrera & Herrera, 1998; Crowther *et al.*, 1999; Hofmeyr *et al.*, 2010; Kanagal *et al.*, 2014). In a prospective study by Levine and colleagues, they noted that calcium supplementation during pregnancy did not prevent preeclampsia in healthy nulliparous women (Levine *et al.*, 1998).

Intracellular calcium concentration determines the tone of vascular smooth muscle and therefore vascular resistance (Cooper, Shamsi, & Katz, 1987). Increased intracellular calcium levels and even increased erythrocyte and platelet ionised calcium levels have been associated with preeclampsia (Haller, Oeney, Hauck, Distler, & Philipp, 1989; Nardulli, Proverbio, Limongi, Marín, & Proverbio, 1994; Sowers *et al.*, 1989). On the contrary, Zemel and colleagues observed no significant difference in intracellular calcium levels in preeclamptics compared with normal pregnant women (Zemel *et al.*, 1990). They noted that intracellular

platelet calcium in preeclamptic women were very sensitive to vasopressin and arginine than those of normal pregnant women and predicted this as a significant factor in the development of preeclampsia (Zemel *et al.*, 1990).

This study reveals no statistically significant difference in the total serum calcium level in the first, second and third trimester groups of the normotensive pregnant women as shown in Figure 4.9. This finding agrees with the studies of Ritchie *et al.*, (1998) but contradicts that of Kovacs & Kronenberg, (1997) who rather noted a significant decrease in total serum calcium as pregnancy progresses.

The finding of no statistically significant difference in total serum calcium levels of normotensive pregnant women as their pregnancy progresses in this study can be explained on the basis that even though calcium utilisation and therefore demand during pregnancy increases, there is a compensatory increase in intestinal calcium absorption and resorption from maternal skeleton (Black, Topping, Durham, Farquharson, & Fraser, 2000; Hellmeyer *et al.*, 2006; Yamaga, Taga, Hashimoto, & Ota, 1999; Yamaga, Taga, & Minaguchi, 1997; Zeni *et al.*, 2003). These compensatory mechanisms have a tendency of maintaining normal or low normal serum calcium levels provided the dietary calcium intake of the mother is not chronically below the minimum recommended daily allowance (<500mg/day) (Ritchie *et al.*, 1998).

Even though there is an increase in calcium demand during pregnancy, the dietary reference intake (DRI) for calcium in 1997 was reduced for pregnant women to equal recommended values for non-pregnant women (Institute of Medicine, 1997). The Institute of Medicine (IOM) committee argued that any calcium deficit not met by an increase in calcium absorption could be provided by resorption of maternal bone calcium. Based on studies by Kalkwarf *et al.*, (1996), it was assumed that maternal bone resorption is recovered by 1 year postpartum. The

most recent IOM report (November 2010) upheld the previous recommendation for dietary calcium intake during pregnancy (Ross *et al.*, 2011).

The increase in maternal calcium absorption as pregnancy progresses is linked to her dietary intake of calcium, with absorption being greatest in pregnant women with low intake (Heaney & Skillman, 1971; Ritchie *et al.*, 1998; Zapata *et al.*, 2004). Several studies have reported that 1,25-dihydroxycholecalciferol levels progressively increase each trimester, hence influencing the raise in calcium absorption (Cross, Hillman, Allen, Krause, & Vieira, 1995; Ritchie *et al.*, 1998). As normal pregnancy progresses, levels of a prohormone, parathyroid hormone receptor protein (PTHrP), rises in maternal circulation (Kovacs, 2005). PTHrP increases both intestinal calcium absorption and renal tubular resorption of calcium (Kovacs & Kronenberg, 1997). Even though there is physiologically a hypercalciuria in pregnancy especially post prandial, this does not offset the absorption rate and therefore there is a net retention of calcium so long as the intake is not chronically below 500mg/day (Bezerra, Mendonça, Lobato, O'Brien, & Donangelo, 2004; Cross *et al.*, 1995; Kovacs & Kronenberg, 1997; Ritchie *et al.*, 1998).

5.5 Serum Sodium Levels and Preeclampsia

From this current study, there was a significant reduction in the mean serum sodium levels in preeclamptic women as compared to normotensive pregnant women as shown in Figure 4.12. Abnormalities of cell membrane transport of sodium causes extravascular retention of sodium with a reduction in plasma sodium levels. There are two mechanisms by which sodium has been proposed to affect blood pressure.

Excessive sodium intake causes sodium and water retention increasing intravascular and extracellular fluid volume. This results in increase venous return and a raised cardiac index with consequent increase in tissue blood flow. As this continues unabated, there is an

autoregulatory mechanism with a consequent increase in total peripheral resistance (Sullivan & Martin, 1994).

The “peripheral arterial vasodilation hypothesis” also postulates an increased endothelial damage with sodium and water retention and hence an increase in sensitivity to angiotensin (Schrier & Briner, 1991).

From this study, there was a significant reduction in the mean serum sodium levels in preeclamptic women as compared to normotensive pregnant as shown in Figure 4.12. This finding is in accordance with those reported by other authors (Indumati *et al.*, 2011; Pitkin, 1972; Searcy, 1969; Tarik & Ward, 2011). However, this finding contradicts the findings of other studies which reported no significant change in serum sodium levels of preeclamptic women as compared to normotensive pregnant women (Adewolu, 2013; Bera *et al.*, 2011; Rizk, 1997; Yussif *et al.*, 2009). Some studies have also noted a statistically significant raised serum sodium levels in preeclamptic women as compared to normotensive pregnant women (Caughey *et al.*, 2005). It can be seen from above that the role of serum sodium in the pathophysiology of preeclampsia still remains unsolved as there seems to be population variations in the findings.

Preeclampsia clinically manifests as hypertension, proteinuria with or without oedema during pregnancy. Even though preeclamptics have an adequate electrolyte and water content, these are mainly situated in the interstitium with a resultant decreased intravascular circulating volume. This decrease in intravascular circulating volume is interpreted by the body's responses as a decreased blood pressure with corresponding activation of baroreceptors and release of antidiuretic hormone (ADH) causing water retention and natriuresis. This sequence of events leads to hyponatraemia and hence the observed significantly reduced serum sodium levels in preeclamptics. A report of 9 cases of hyponatraemia in preeclampsia has been

documented in literature (Ravid *et al.*, 2005). It has also been observed that a defective placenta seen in preeclampsia is unable to produce a vasopressinase, an enzyme which inactivates ADH and therefore a build-up of ADH in preeclampsia (Chung, Kluge, Schrier, & Anderson, 1987).

Alteration of natriuretic factors as being responsible for hyponatraemia seen in preeclampsia has also been suggested in literature. Atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) levels has been noted to be increased in preeclamptic women even though this is not a uniform finding (Graves, 2007; Reis *et al.*, 2003; Tihtonen, Kööbi, Vuolteenaho, Huhtala, & Uotila, 2007). These factors cause natriuresis and hence a fall in serum sodium levels. This study however did not measure the levels of natriuretic factors.

There are also reports of dilutional hyponatraemia in preeclamptic women with or without associated nephrotic syndrome in literature (Hayslett, Katz, & Knudson, 1998; Magriples, Laifer, & Hayslett, 2001). Preeclampsia is one of the commonest causes of nephrotic syndrome that occurs de novo during pregnancy (Fisher, Ahuja, Luger, Spargo, & Lindheimer, 1977). There is a difficulty distinguishing between preeclampsia and renal disease as a cause of hyponatraemia in this case. The distinction is usually possible in retrospect, as clinical signs of preeclampsia generally resolve within 12 weeks postpartum, while proteinuria due to underlying renal disease does not (Chua & Redman, 1992).

From this current study, there was a significant difference between the mean serum sodium levels among the first and second trimester normotensive pregnant women, first and third trimester normotensive pregnant women but a statistically non-significant difference between the second and third trimester normotensive pregnant women as shown in Figure 4.13. A significant drop in serum sodium was noticed in the first trimester. It has been suggested that plasma threshold for release of ADH and thirst stimulation are lowered during pregnancy. This is made possible by the effect of an increased β -human chorionic gonadotropin (Davison,

Shiells, Philips, & Lindheimer, 1988). This occurrence in causing a reduction in serum sodium levels is also believed to be related to vasodilatation and poor arterial filling (Schrier, 2010). Studies have shown that systolic, diastolic and mean arterial blood pressure progressively decreases from the beginning of pregnancy reaching a statistically significant trough around 22 weeks of gestation after which it rises progressively till term (Grindheim *et al.*, 2012). This progressive reduction in blood pressure results in reduced effective circulating volume and a relative arterial underfilling. Subsequent to these alterations there is a release of non-osmotic arginine vasopressin (AVP) with resultant sympathetic and renin-angiotensin stimulation and therefore reduced plasma osmolarity and serum sodium levels. The mechanism initiating the above process has been suggested to be the presence of the fetoplacental unit (Lindheimer & Davison, 1995). However, there is also a significant accumulation of sodium as a result of a rise in aldosterone and deoxycorticosterone which are anti-natriuretic. Sodium accumulates despite a rise in glomerular filtration rate (GFR) which causes natriuresis. Sodium and water retention is important in offsetting the increased maternal extracellular compartment and also meeting foetal demands (Sturgiss, Dunlop, & Davison, 1994).

5.6 Serum Potassium Levels and Preeclampsia

From the current study, there was a significant reduction in mean serum potassium levels of preeclamptic women as compared to normotensive pregnant women as shown in Figure 4.14. Manjareeka & Nanda, 2012; Yussif *et al.*, 2009 made a similar observation in an Indian and Iraqi population respectively while other studies (Adewolu, 2013; Bera *et al.*, 2011; Siddiqui & Rana, 1993; Singh *et al.*, 1993) observed no statistically significant difference in the serum potassium level among hypertensive pregnant women and normotensive pregnant women in India. However, Handwerker and colleagues (1995) also reported a statistically significant elevated serum potassium level in preeclamptic women as compared to normotensive pregnant women.

Ninety percent (90%) of total body potassium is located intracellularly whilst sodium is mainly located extracellularly. The difference in predominant locations is influenced by the Na^+/K^+ -ATPase (Delgado, 2004). In preeclampsia and pregnancy induced hypertension there is an abnormality in the transport of sodium and potassium across the cell membrane of vascular smooth muscles which regulates blood pressure (Arumanayagam & Rogers, 1999). Earlier studies (Pikilidou *et al.*, 2007) have conclusively established an inverse relationship between serum potassium levels and severity of hypertension. This shows that raised serum potassium may have a beneficial effect on blood pressure regulation. The mechanism explaining the relationship between the reduced serum potassium levels and poor blood pressure control is poorly understood. Reduced levels of serum potassium enhance vascular responsiveness to vasopressors such as norepinephrine (Bianchetti, Weidmann, Beretta-Piccoli, & Ferrier, 1987). Decreased release of nitric oxide by the endothelial cells may be the mediating factor (Taddei *et al.*, 1994). This leads to vasoconstriction and increased platelet aggregation which increases blood pressure and therefore poor protection against hypertension caused by induced endothelial injury which is responsible for the pathogenesis of preeclampsia (Vane, Änggård, & Botting, 1990).

It has also been observed that reduced serum potassium levels also reduce sodium excretion through probable changes in reabsorption of sodium in the proximal tubule or loop of Henle of the kidney resulting in elevated blood pressure (Gallen *et al.*, 1998). In fact it has been observed that a diet low in potassium coupled with individual's usual sodium intake can lead to sodium retention and therefore hypertension (Yussif *et al.*, 2009).

Another finding from this study is an increase in serum potassium levels among normotensive pregnant women compared with normotensive non-pregnant women though statistically not significant as shown in Figure 4.14. This finding agrees with that of other authors (Anastasiadis & Rimpler, 1984). Total body potassium stores increases as gestation progresses (Gonzalez-

Campoy, Romero, & Knox, 1989; Tkachenko, Shchekochikhin, & Schrier, 2014). This increase occurs despite the sodium retention from aldosterone because of the antikaliuretic effects of progesterone. Progesterone levels increase progressively from early pregnancy till term (Lindberg, Nilsson, & Johansson, 1974). Potassium excretion is however kept constant throughout pregnancy, with changes in tubular reabsorption adapting to alterations in filtered load (Cheung & Lafayette, 2013).

This study reveals a statistically significant difference between the serum potassium levels among the first and third trimester normotensive pregnant women as shown in Figure 4.15. There is a progressive increase in serum potassium levels from first through to the third trimester. This finding is consistent with the findings of Tomala and colleagues (1994) who noted a progressive rise in serum potassium levels as pregnancy progresses. This can be explained based on the progressive increase in progesterone levels noticed during normal pregnancy (Lindberg *et al.*, 1974). Progesterone causes stimulatory effect on potassium and hydrogen transport respectively by stimulating the $H^+-K^+ \alpha_2$ -ATPase isoform in the distal nephron with resultant positive potassium balance (Elabida *et al.*, 2011; Lindheimer, Richardson, Ehrlich, & Katz, 1987).

5.7 Serum Nitric Oxide Levels and Preeclampsia

From this current study, there was a statistically no significant reduction in serum nitric oxide levels in preeclampsia compared to normotensive pregnant women. This finding agrees with the findings of Monaghan (1999). However, there was a statistically significant elevation in serum nitric oxide levels in preeclampsia as compared to normotensive non-pregnant women as shown in Figure 4.14. There was also a statistically significant elevation in serum nitric oxide levels among normotensive pregnant women as compared to normotensive non-pregnant women.

Nitric oxide is a chemical messenger involved in neuronal transmission, vascular homeostatic regulation and immune system control. Nitric oxide has been postulated to have several functions in normal pregnancy including decreased peripheral vascular resistance maintenance, prevention of platelet aggregation in fetoplacental circulation, uterine relaxation and antagonising vasopressor effects.

Previous studies designed to search for a relationship between nitric oxide production in preeclampsia and normal pregnancy has shown inconsistent conclusions. Various researchers have noted an increase in nitric oxide levels in preeclampsia as compared to normotensive pregnant women (Davidge *et al.*, 1996; Nobunaga *et al.*, 1996; Norris *et al.*, 1999; Smáráson *et al.*, 1997). Other researchers have also found a decrease in serum nitric oxide levels in preeclamptic patients compared to normotensive pregnant women (Diejomaoh *et al.*, 2004; Garmendia *et al.*, 1997; Seligman *et al.*, 1994; Silver *et al.*, 1996). The proponents for increased nitric oxide levels in preeclampsia compared to normotensive pregnant women argue that the increase is as a result of a compensatory mechanism for the occurring endothelial damage in preeclampsia hence an attempt to correct the vasospasm effect. However, the proponents for decreased nitric oxide levels in preeclamptic as compared to normotensive pregnant women suggest that the reduction is as a result of a down regulation of the nitric oxide synthase enzyme and/or occurrence of endothelial damage in the development of the disorder. The results of a statistically non-significant difference in serum nitric oxide levels among preeclamptic and normotensive pregnant women in this study is supported by many others (Conrad & Mosher, 1995; Curtis *et al.*, 1995; Di Iorio *et al.*, 1998; Lyall *et al.*, 1995; Monaghan, 1999). This finding however does not mean that there is no association between serum nitric oxide levels and preeclampsia but then as per this study, the change in nitric oxide level cannot explain the pathophysiology of preeclampsia. This can be explained on the basis that determination of serum nitric oxide levels is confounded by several factors including diet, alcohol consumption,

atmospheric pollution, exercise and cigarette smoking (Baylis, Suto, & Conrad, 1996; Sooranna, Morris, & Steer, 1995). Significantly increased nitric oxide production has been associated with several disease states (Cunha, Moncada, & Liew, 1992; Nava, Palmer, & Moncada, 1991; Vora, Monaghan, Nuttall, & Crowther, 1997). In disorders where there may be a very small difference in nitric oxide production, it may be impossible to find a significant change over the uncontrolled external factors stated above. Considering the study site, it may be very difficult eliminating the above inter-subject variations and therefore a longitudinal study on selected subjects where inter/intra personal variations can be seen as well as any differences in nitric oxide levels throughout pregnancy between preeclamptic and normotensive pregnant women is advised. Other studies have also admitted the contradictory reports regarding the involvement of nitric oxide in maternal adaptation to pregnancy and suggested a possible multi-mechanism physiology acting in concert to maintain the pregnant mother and the foetus with the input from each mechanism being genetically determined (Buhimschi *et al.*, 1998).

The significant elevation in nitric oxide levels in normotensive pregnant women compared to normotensive non-pregnant women (Choi, Im, & Pai, 2002; Shaamash *et al.*, 2000) may be due to the profound increase in intravascular volume associated with normal pregnancy. There is a drop in blood pressure in normal pregnancy due to decreased peripheral resistance secondary to extensive systemic vasodilatation. Nitric oxide being a potent vasodilator may mediate this effect (Meher & Duley, 2007).

In the study, serum nitric oxide levels increased progressively with advancing gestational age as shown in Figure 4.15. This increase was however statistically non-significant. This finding has also been noted in literature (Choi, Im, *et al.*, 2002; Jo *et al.*, 1998; Shaamash *et al.*, 2000). These studies showed that serum nitric oxide levels increase with advancing gestation peaking

in the third trimester of normal pregnancy. Varied conclusions however have been reported in other studies (Brown, Tibben, Zammit, Cario, & Carlton, 1995; Hata *et al.*, 1999).

It is therefore clear from above that serum nitric oxide status in normotensive pregnant women still remains controversial. These inconsistencies may be as a result of non-uniformity in assaying methods for measuring serum nitric oxide levels. Some of these assaying methods may incompletely measure circulating nitric oxide metabolites and hence a source of variability. Also, dietary nitrate and iron influences serum levels of nitric oxide metabolites. Various studies have correlated iron deficiency anaemia with an increase in serum nitric oxide levels in pregnancy (Choi, Pai, *et al.*, 2002; Mabbott & Sternberg, 1995; Ni, Morcos, & Vaziri, 1997). Since iron deficiency anaemia is quite common in advancing pregnancy especially in the study setting, it may account for the increasing nitric oxide levels as gestation progresses (Figure 4.15). However, it is also noted that metabolites of nitric oxide can be influenced by renal clearance as well as production (Conrad, Kerchner, & Mosher, 1999).

The finding from this study and other previous studies clearly prove inconsistent results concerning serum nitric oxide levels in normal pregnancy and preeclampsia. Whichever the pathogenesis is in preeclampsia, it always points to the fact that nitric oxide is involved in endothelial cell dysfunction in preeclampsia which is stated in all literatures concerning nitric oxide and preeclampsia.

CONCLUSIONS

There was a progressive reduction in the mean arterial pressure from the first to the second trimester and increased thereafter in the third trimester while there was a progressive increase in the mean heart rate from the beginning of pregnancy till term in normotensive pregnant women.

There was a statistically significant reduction in serum magnesium, sodium and potassium levels in preeclampsia compared to normotensive pregnant women.

There was a statistically non-significant reduction in serum total calcium, ionised calcium and nitric oxide levels in preeclampsia compared to normotensive pregnant women.

There was no statistically significant difference in the mean serum magnesium, total calcium and nitric oxide levels between the first, second and third trimester normotensive pregnant women.

A statistically significant difference between the serum ionised calcium, sodium and potassium levels in first, second and third trimester normotensive pregnant women.



RECOMMENDATIONS

Longitudinal studies with large sample sizes where women with early pregnancy are recruited and confounding factors like diet controlled and followed up till term with serial measurement of their serum electrolyte and nitric oxide and also identifying those that will develop preeclampsia is advised.

A study on the predictive factors of the above measured serum electrolytes in preeclampsia and normal pregnancy will help better understand the pathophysiology of electrolytes and nitric oxide in preeclampsia.

LIMITATIONS OF THE STUDY

The study did not measure urinary excretion of magnesium, calcium, sodium, potassium and nitric oxide and relate it to renal clearance as this can affect the serum levels of the above markers.



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APPENDIX

Appendix 1: Informed Consent Form

Dear Client,

My name is Ebenezer Owusu Darkwa. I am a student at the Department of Physiology at the School of Biomedical and Allied Health Sciences, University of Ghana.

Study title

I am conducting a study titled “Electrolyte Physiology of Selected Serum Electrolytes and Nitric Oxide in pre-eclamptic women at Korle- Bu Teaching Hospital, Ghana.”

Aims

The main aim of the study is to determine the serum levels of above markers in healthy pregnant women and pre-eclamptic women and how this affects the development of hypertension in these patients.

Background

Serum electrolyte and nitric oxide levels of pre-eclamptic women and healthy pregnant women attending antenatal clinic at Korle-Bu Teaching Hospital is unknown. However, literature has shown an association between the development of pre-eclampsia and serum electrolytes/nitric oxide levels in pregnant women.

Description of procedure

If you agree to participate in the study, you will have your antenatal clinics done normally. Your demographic details including your age, weight, height, body mass index (BMI), parity and educational status will be noted. Your blood pressure and heart rate will also be recorded. A blood sample will be taken from you for laboratory analysis of its serum electrolyte/nitric oxide levels.

Risks

The risk this study could pose to you is from the venepuncture you will receive when blood samples are being taken for laboratory analysis. The problems with the venepuncture could be bleeding, pain, and transmission of infection. However, selected patients for the study will have an EMLA patch (local anaesthetic patch) before the venepuncture to reduce pain. The venepuncture process will also be done aseptically by cleaning the skin with 70% alcohol

therefore preventing transmission of infection. Pressure and a small adhesive patch will be applied to site of venepuncture to prevent and manage any bleeding that occurs.

Benefits

All patients recruited in the study will have their serum magnesium, nitric oxide, sodium, calcium and potassium levels checked free of charge for them.

Assurance of confidentiality

Your data collected will be kept confidential. Information derived from the study will be used to improve management of pregnant women.

Explanation

You reserve the right to refuse to participate in this study, and that will not affect how we treat you as our client. We need you to consent if you are happy with our explanation and will like to participate in the study.

Many thanks.

.....

Signature/ thumbprint of client/ Date	Signature of investigator/Date (0244670149)
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.....

Signature of witness/Date



Appendix 2: Data Collection Sheet

The Role of Some Selected Electrolytes and Nitric Oxide in The Pathophysiology of Preeclampsia in Ghanaian Women

Date: Patient's identification number (ID):

Category: A. Normotensive pregnant women [] B. Preeclamptic women []

C. Normotensive non-pregnant women []

Demographic Parameters

1. Age: (years)
2. Weight: (kg)
3. Height: (cm)
4. BMI: (kg/m²)
5. Tribe:
6. Gestational age: [0-13 Weeks] [14-28 Weeks] [29-40+ Weeks]
7. Parity:
8. Educational status: [None] [Primary] [Secondary] [Tertiary]

Physical Assessment

9. First Blood Pressure:(mmHg)
10. Second Blood Pressure (after 15minutes):(mmHg)
11. Average Blood Pressure: (mmHg)
12. First Heart Rate: (bpm)
13. Second Heart Rate (after 15 minutes): (bpm)
14. Average Heart rate: (bpm)

Laboratory Parameters

15. Serum Magnesium Level:(mmol/L)
16. Serum nitric oxide level:(micromol/L)
17. Serum calcium level:(mmol/L)
18. Serum sodium level:(mmol/L)
19. Serum potassium level:(mmol/L)

Appendix 3: Comparison of the means of demographic, clinical and basic laboratory data for preeclamptic, normotensive pregnant and normotensive non-pregnant women (Table 4.1)

Pairwise comparison analysis: post hoc analysis of group difference were as follows:

I. Height

a. Preeclampsia × Normotensive pregnant	p-value = 0.036
b. Preeclampsia × Normotensive non-pregnant	p-value = 0.040
c. Normotensive pregnant × Normotensive non-pregnant	p-value = 0.870

II. Parity

a. Preeclampsia × Normotensive pregnant	p-value = 0.008
b. Preeclampsia × Normotensive non-pregnant	p-value = 0.363
c. Normotensive pregnant × Normotensive non-pregnant	p-value = 0.349

III. Systolic Blood Pressure

a. Preeclampsia × Normotensive pregnant	p-value = 0.000
b. Preeclampsia × Normotensive non-pregnant	p-value = 0.000
c. Normotensive pregnant × Normotensive non-pregnant	p-value = 0.236

IV. Body Mass Index (BMI)

a. Preeclampsia × Normotensive pregnant	p-value = 0.003
b. Preeclampsia × Normotensive non-pregnant	p-value = 0.108
c. Normotensive pregnant × Normotensive non-pregnant	p-value = 0.646

V. Heart Rate (HR)

a. Preeclampsia × Normotensive pregnant	p-value = 0.001
b. Preeclampsia × Normotensive non-pregnant	p-value = 0.004
c. Normotensive pregnant × Normotensive non-pregnant	p-value = 0.919

VI. Mean Arterial Pressure (MAP)

a. Preeclampsia × Normotensive pregnant	p-value = 0.000
b. Preeclampsia × Normotensive non-pregnant	p-value = 0.000
c. Normotensive pregnant × Normotensive non-pregnant	p-value = 0.012

VII. Serum Creatinine

a. Preeclampsia × Normotensive pregnant	p-value = 0.008
b. Preeclampsia × Normotensive non-pregnant	p-value = 0.973
c. Normotensive pregnant × Normotensive non-pregnant	p-value = 0.014

VIII. Serum Urea

a. Preeclampsia × Normotensive pregnant	p-value = 0.189
b. Preeclampsia × Normotensive non-pregnant	p-value = 0.000
c. Normotensive pregnant × Normotensive non-pregnant	p-value = 0.000

IX. Haemoglobin Concentration (HB)

a. Preeclampsia × Normotensive pregnant	p-value = 0.844
b. Preeclampsia × Normotensive non-pregnant	p-value = 0.159
c. Normotensive pregnant × Normotensive non-pregnant	p-value = 0.013

X. Haematocrit (HCT)

a. Preeclampsia × Normotensive pregnant	p-value = 0.805
b. Preeclampsia × Normotensive non-pregnant	p-value = 0.258
c. Normotensive pregnant × Normotensive non-pregnant	p-value = 0.026

XI. ALT

a. Preeclampsia × Normotensive pregnant	p-value = 0.000
b. Preeclampsia × Normotensive non-pregnant	p-value = 0.103
c. Normotensive pregnant × Normotensive non-pregnant	p-value = 0.323

XII. AST

- | | |
|--|-----------------|
| a. Preeclampsia × Normotensive pregnant | p-value = 0.000 |
| b. Preeclampsia × Normotensive non-pregnant | p-value = 0.002 |
| c. Normotensive pregnant × Normotensive non-pregnant | p-value = 0.949 |

XIII. Serum Total Protein

- | | |
|--|-----------------|
| a. Preeclampsia × Normotensive pregnant | p-value = 0.948 |
| b. Preeclampsia × Normotensive non-pregnant | p-value = 0.046 |
| c. Normotensive pregnant × Normotensive non-pregnant | p-value = 0.022 |

XIV. Direct Bilirubin (B Direct)

- | | |
|--|-----------------|
| a. Preeclampsia × Normotensive pregnant | p-value = 0.016 |
| b. Preeclampsia × Normotensive non-pregnant | p-value = 0.743 |
| c. Normotensive pregnant × Normotensive non-pregnant | p-value = 0.130 |



Appendix 4: Comparison of the means of serum electrolytes and nitric oxide of preeclamptic, normotensive pregnant and normotensive non-pregnant women (Table 4.2)

Pair wise comparison analysis: post hoc analysis of group differences were as follows:

I. Serum Sodium

a. Preeclampsia × Normotensive pregnant	p-value = 0.006
b. Preeclampsia × Normotensive non-pregnant	p-value = 0.106
c. Normotensive pregnant × Normotensive non-pregnant	p-value = 0.805

II. Serum Potassium

a. Preeclampsia × Normotensive pregnant	p-value = 0.011
b. Preeclampsia × Normotensive non-pregnant	p-value = 0.426
c. Normotensive pregnant × Normotensive non-pregnant	p-value = 0.324

III. Serum Nitric Oxide

a. Preeclampsia × Normotensive pregnant	p-value = 0.447
b. Preeclampsia × Normotensive non-pregnant	p-value = 0.001
c. Normotensive pregnant × Normotensive non-pregnant	p-value = 0.000



Appendix 5: Comparison of the means of serum electrolytes of first, second and trimester normotensive pregnant women (Table 4.3)

Pair wise comparison analysis: post hoc analysis of group differences were as follows:

I. Serum ionised calcium of normotensive pregnant women

- | | |
|---------------------------------------|-----------------|
| a. First trimester × Second trimester | p-value = 0.000 |
| b. First trimester × Third trimester | p-value = 0.023 |
| c. Second trimester × Third trimester | p-value = 0.334 |

II. Serum potassium of normotensive pregnant women

- | | |
|---------------------------------------|-----------------|
| a. First trimester × Second trimester | p-value = 0.088 |
| b. First trimester × Third trimester | p-value = 0.000 |
| c. Second trimester × Third trimester | p-value = 0.099 |

