Serum Minerals Levels in Pre-eclampsia and Eclampsia: Association with clinical complications

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Declaration

This is to certify that the thesis titled, "**Serum Minerals levels in Pre-eclampsia and Eclampsia: Association with clinical complications**" submitted by **Salina Banu** of registration no 354, session 1999-2000 for the degree of **Master of Philosophy**, University of Dhaka is a record of original research work. Salina Banu has carried out this research work under our supervision and guidance at the Institute of Nutrition and Food Science, University of Dhaka. The results used in this thesis have not been submitted elsewhere for the award of any other degree.

This thesis is worthy for the award of the degree of "**Master of Philosophy**" in accordance with the rules and regulation of Dhaka University.

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DEDICATED TO MY MOTHER

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Abstract

The study investigated mineral profile (Ca, Mg, Cu, Zn, Fe) of pre-eclampsia and eclampsia and attempted to find association of the mineral to the aetiology of the complication. It was a case control study conducted on 33 pre-eclampsia and 44 eclampsia case subjects and 27 normotensive pregnants taken as pregnant control. Twenty nine normotensive non-pregnant women were also included to determine the basal mineral status. The case and control subjects were recruited under defined criteria. Ethical permission was taken from the departments concerned for recruitment of the patients. Serum mineral concentration was analyzed by Atomic Absorption Flamme Spectrophotometer equipped with a hollow cathode lamp and a deuterium background corrector at respective wavelengths using an air-acetylene flame. SPSS (12.5 version) software package was used for statistical analysis.

Results showed that there was significant ($p<0.05$) increase in serum concentrations of Ca and Mg in eclampsia as compared to those in pre-eclampsis, but concentrations of Cu, and Zn in pre-eclamsia were higher than those in eclampsia. Calcium and magnesium concentrations in eclampsia were found significantly ($P<0.05$) high as compared to those in the pregnant control, but in case of copper it is reverse. All of the mineral values in the study women were found to be within the normal range. However, the serum magnesium level was noted in the lowest limit. Copper to iron ratio was found significantly high in eclampsia as compared to that in other subjects. Pearson correlation analysis indicated a significant ($p<0.05$) positive correlation in serum magnesium change with zinc in eclampsia and magnesium change with iron in both the pre-eclampsia and eclampsia. It may be concluded that change in mineral value in pre-eclampsia and eclampsia may have implication on the pathogenesis of this pregnancy complications.

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CHAPTER 1
INTRODUCTION

1. Introduction

Preeclampsia is one of the most common causes of maternal and fetal morbidities and mortalities (ACOG, 2002). Eclampsia is primarily a convulsive state, despite extensive physiological, biochemical and anatomical changes that occur during pregnancy and may be local or systemic. Pre-eclampsia and eclampsia is a transient but potentially dangerous complication of pregnancy that affects 3-5% of pregnancies (Skjaerven et al, 2002; Sarsam et al, 2008). Eclampsia is commonly seen in teenage pregnant woman who lives in slum area devoid of both home care and antenatal care. In Bangladesh, eclampsia accounts five percent of total obstetric admission in our health facilities and sixteen percent of maternal death (Sultana et al, 2003). Pregnancy is associated with increased demand of all the nutrients including iron, copper, zinc, vitamin B_{12}, folic acid and ascorbic acid (Naeye et al, 1993) and deficiency of any of these could affect pregnancy, delivery and outcome of pregnancy. On the physiological basis, calcium plays an important role in muscle contraction and regulation of water balance in cells. Modification of plasma calcium concentration leads to the alteration of blood pressure. The lowering of serum calcium and the increase of intracellular calcium can cause an elevation of blood pressure in preeclamptic mothers. The serum magnesium also decreases in women with preeclampsia (Kisters et al, 2000; Ray et aj, 1999). Generally, magnesium has been known as an essential cofactor for many enzyme systems. It also plays an important role in neurochemical transmission and peripheral vasodilatation. Magnesium sulfate appears to be safe and effective for the prevention of seizures and has been used as the drug of choice in severe preeclampsia and eclampsia treatment (Walker, 2005, Sing et al, 2005). Besides the serum calcium and magnesium, the hyperuricemia is believed to result from the decreased renal excretion that occurs as a consequence of the preeclampsia but this result is probably also increased production of secondary to tissue ischemia and oxidative

stress. Though the cause of eclampsia is unknown but eclampsia is now considered as malnutrition related disease (Chowdhury, 2007). Malnutrition is common in our country. Pregnancy imposes a great stress on the nutritional reserves. There is depletion of essential nutrients like vitamin- B complexes, vitamin-A, folic acid, iron and calcium. Intake of the essential nutrient in poor income women is far below than recommended daily allowances. Decreased protein intakes causes decrease calcium absorption from the gut and stimulate parathyroid hormone secretion, and thus enhance calcium reabsorption from bone and maintain calcium balance (Joel et al, 1994). There is progressive decline in magnesium consumption from 475-500mg/day in 1900—1908 to 175-225mg/day in 1990-2002 due to widespread consumption of processed foods and decreased consumption of fresh foods. Nuts and green leafy vegetables are good sources of magnesium (LewRockwell.com 2002). Serum electrolytes and ionized Ca^{2+} level remain normal (William et al, 1994; Dutta, 2005; Rashid et al, 1996). It has been dubbed the "disease of theories" because of the multiple hypotheses have been proposed to explain its occurrence (Solomon 2004). In spite of numerous studies, the etiology of pre-eclampsia has not been fully elucidated. Some studies have concluded that changes in levels of blood metals observed in preeclamptic patients may implicate the pathogenesis of pre-eclampsia (James et al, 2006; Bringman et al, 2006). Other studies have failed to show an association between the serum concentrations of these elements and occurrence of pre-eclampsia (Gabbeet al, 2002; Cunningham et al, 2005). High rate of pre-eclampsia in developing countries have forced some authors to conclude that malnutrition is a risk factor in the etiology of pre-eclampsia and implicate it by deficit intake of calcium and zinc (Caughey et al, 2005). Other studies claim that blood calcium and magnesium have a relaxant effect on the blood vessels of pregnant women. This is the first study that measures serum concentrations of five elements simultaneously in pre-eclamptic and eclamptic women.

1.1 Rational of the study

Pre-eclampsia along with its complications eclampsia seems to be one of the major causes of maternal morbidity and mortality. Its greatest impact is in the developing countries accounting for 20-80% of the increased maternal death. Even in the developed countries there is major effect, primarily on the foetus. Eclampsia is commonly seen in teenage pregnant woman who lives in slum area devoid of both home care and antenatal care. In Bangladesh, eclampsia accounts five percent of total obstetric admission in our health facilities and sixteen percent of maternal death. Despite numerous studies, the etiology of preeclampsia has not yet been fully elucidated. Minerals contribute to development of hypertension. Role of micronutrients (vitamins and minerals) in regulation of blood pressure have been explored by several investigators (Dakshinamuri and Dakshijnamuri, 2001). The steady-state concentrations of Ca and Ma are reciprocally related. The role of Ca in regulating cellular processes including vascular tone, is dependent upon (and modified by) cellular Mg. Intracellular Mg deficiency has been reported to induce hypertension. Elevation of Ca^{2+} ion with a reciprocal decrease in Ma^{2+} ion is consistently present in hypertension. Resnick (1999) reviewed that Ca deficiency and Ca excess contributed to development of hypertension and hypothesized that ionic defect induces cellular defects resulting in a variety of related clinical abnormalities.

Although a number of researches into the aetiology and mechanism of preeclampsia has been documented, its exact pathogenesis still remains uncertain. Some studies reported that changes in the levels of blood trace elements in pre-eclamptic patients may implicate its pathogenesis (Bringman et al, 2006; James et al, 2006) while others have failed to show an association of blood levels of trace elements and prevalence of pre-eclampsia (Caughey et al, 2005). In Bangladesh, pre-eclampsia and eclampsia cause high maternal and perinatal morbidity and mortality. It is thought that change in or deficiency of mineral in pregnancy may be associated with

the complication of preeclampsia and eclampsia. The present study has, therefore, attempted to analyze serum mineral profile of pre-eclampsia and eclampsia and to address influence or association of the minerals with the complications of preeclampsia and eclampsia.

Finding of this study would help prevention and management of this pregnancy related complication, and thus would support the health care facilities. In addition, this study would shed some light in the unclarified aetiology of pre-eclamsia and eclampsis, particularly with respect to changes in mineral profile in the patients.

1.2 Objective

Aims of the study are -

i. to investigate serum minerals (Ca, Mg, Zn, Cu, Fe,) concentrations of pre-eclampsia and eclampsia subjects.

ii. to assess the influence of or relation to or association of serum minerals with the preeclampsia and eclampsia subjects

1.3 Hypothesis

Changes in serum mineral profile are associated in the development of eclampsia and preeclampsia.

CHAPTER 2
REVIEW OF LITERATURE

2. Review of Literature

The development, in the later half of pregnancy, of new-onset hypertension and proteinuria that resolves postpartum– pre-eclampsia, it is a potentially life threatening condition. It appears to be a disease unique to human pregnancies. It is an enigmatic condition because in spite of rigorous investigation, the underlying aetiology remains obscure. This thesis describes new investigations into the aetiology of pre-eclampsia using molecular genetic and epidemiological methods in the Sinhalese in the Sri Lankan population and attempts at replicating the results in the white Caucasians in Nottingham, UK

2.1 The definition

The definition of pre-eclampsia has been the subject of much debate. Greater appreciation of the underlying pathophysiological changes of the disease, however, has enabled different expert groups working independently of each other to arrive at almost uniform criteria for its definition reflecting the known pathophysiology of the disorder (Brown et al, 2000). The International Society for the Study of Hypertension in Pregnancy (ISSHP) defines pre-eclampsia for research purposes as *de novo* hypertension (systolic blood pressure (SBP) ≥140 mmHg and diastolic blood pressure (DBP) ≥ 90 mmHg) after 20 weeks of gestation in a previously normotensive pregnant woman, which returns to normal by the end of the third month postpartum, together with properly documented proteinuria (≥300 mg/L in a 24 hour collection or ≥500mg/L on a spot urine collection or ≥30 mg protein/mmol creatinine in a spot urine collection or, failing other measurement, ≥1+ on dipstick testing) not associated with urinary tract infection or ruptured membranes (Brown et al, 2001). The presence of edema, which was used in the past as a necessary feature of the diagnosis of pre-eclampsia, has now been dropped from both clinical and research definitions because it is not specific to the condition.

2.1.1 The burden of Preeclampsia and eclampsia

Pre-eclampsia is a major medical problem in pregnancy throughout the world. To appreciate the burden of this problem, it has to be considered in the wider context of the spectrum of hypertensive disorders of pregnancy: Gestational Hypertension (GH), Pre-eclampsia (PE), a majority of cases of eclampsia (E) and HELLP Syndrome (Hemolysis, Elevated Liver enzymes, Low Platelet count in association with hypertension). Gestational hypertension is the occurrence of *de novo* hypertension in pregnancy. Eclampsia is the occurrence of generalized convulsions during pregnancy, labour, or within seven days of delivery which is not caused by epilepsy or other convulsive disorders. The HELLP syndrome, the definition of which may vary slightly from country to country, is usually indicated by the presence of an abnormal peripheral blood smear, with schistocytes and/or burr cells; serum lactate dehydrogenase, bilirubin, and transaminase more than twice the upper limit of normal, and platelet count less than $100x10^9$/L.

2.1.2 Incidence of preeclampsia disorders of pregnancy

There are many reports on the occurrence of hypertensive disorders of pregnancy in different parts of the world. Very few of these, however, could be used to estimate their incidence with any degree of reliability and an even smaller number could be used for comparison. The reported incidence shows great variation. This may be attributable to differences in definition, population composition, demographic and obstetric characteristics, actual disease incidence, or access to and availability of antenatal care services (WHO, 1987). A population based international collaborative study designed to control for these factors found that clinically recognized hypertension during pregnancy varied by a factor of 25 (Incidence range 1.2% to 31.0%) between countries. Even using a strict definition of proteinuric hypertension, the incidence varied by a factor of five (incidence range 1.5% to 8.3%) (WHO,1988).

2.1.3 Morbidity and mortality associated with hypertensive disorders of pregnancy

In the developed world, the syndrome of pre-eclampsia and eclampsia is one of the two most common causes of maternal mortality. However, it is the developing world, where pre-eclampsia is the third commonest cause of maternal death behind hemorrhage and infection (WHO, 1999), that accounts for the overwhelming majority of the estimated 50,000 annual maternal deaths from hypertensive disorders of pregnancy. In Africa, Latin America, and the Caribbean, pre-eclampsia and eclampsia are estimated to account for 20-25% of maternal deaths (Duley, 1992).

2.1.4 Recurrence of pre-eclampsia in subsequent pregnancies

The repeated occurrence of pre-eclampsia or gestational hypertension in several pregnancies of the same woman is not a rare event. The recurrence of pre-eclampsia or gestational hypertension in subsequent pregnancies in some studies has been as high as 50%. Some retrospective studies estimating the recurrence risks of pre-eclampsia, however, may not give the true picture, as they have not been able to verify the diagnosis by examining case notes since strict criteria may not have been applied to diagnose the condition in the past. Indeed in some studies, including that of long term studies by Chesley et al (1976); 'eclampsia' has been used as a surrogate for the diagnosis of pre-eclampsia as the former was likely to be more accurate. A previous normal pregnancy, and to some extent even a previous abortion, is associated with a markedly lowered incidence of pre-eclampsia (Dekker et al, 1998). The protective effect of multiparity is lost, however, with a change of partner (Robillard et al, 1993). Robillard *et al* coined the word primipaternity to describe this phenomenon, suggesting that preeclampsia might be a problem of primipaternity rather than primigravidity because the patterns of changing paternity were significantly correlated with pre-eclampsia in multivariate (Robillard et al, 1999). In contrast to these findings, two groups who analysed the data from the Medical

Birth Registry of Norway covering all births in that country from 1967 to 1998, independent of each other, concluded that a change of partner may in fact decrease the risk of pre-eclampsia in a subsequent pregnancy (Trogstad et al, 2001). This casts a strong element of doubt on the entire concept of primipaternity. In both these studies the risk of pre-eclampsia was shown to increase as the interval between pregnancies became longer. Skjaerven et al (2002) reported that this increased risk applies to all women, but Trogstad et al (2001) reported that it applies only to women who were normotensive in their first pregnancy and observed that the risk of pre-eclampsia for women who have had pre-eclampsia in their first pregnancies reduced with longer inter nine pregnancy intervals. However, these registry based epidemiological studies are prone to error.

2.1.5 Long term sequelae of pre-eclampsia

Chesley et al (1976) who followed up a group of women with eclampsia delivering in the period 1931 through 1951, upto1973-74 were the first to provide definitive data on the possible long term sequelae of eclampsia. The results did not find an excess rate of hypertension or an increase in cardiovascular morbidity or mortality in general in women who had eclampsia only in their first pregnancy. Women who had eclampsia as multivariate, however, had a greater incidence of hypertension as well as higher death rates from all causes, specifically cardiovascular disease later in life. Fisher et al (1981) reported similar results in a group of women with preeclampsia. The result also showed that an age and race matched group of women who were normotensive during pregnancy were at a significantly lower risk of 10 developing hypertension later in life than the population at large. These findings suggested that pre-eclampsia/eclampsia in second or subsequent pregnancy may unmask hypertension in women who are destined to have chronic hypertension later in life and that they are at greater risk of these complications than women in the population at large. Some of these early findings, however, are in conflict with more recent data

(Sibai, 2002). There seems to be agreement on the finding that women who have normotensive pregnancies are at a lower risk of long-term hypertension than the general female population of a similar age. Women who develop preeclampsia in their first pregnancy, however, seem to be at a higher risk of hypertension later in life and women who have pre-eclampsia/eclampsia in more than one pregnancy are at still greater risk (Sibai, 2002; Martinez-Abundis et al, 2000). Although Martinez-Abundis et al. (2000) reported, in agreement with Chesley et al (1976), that eclampsia is not associated with a greater risk of long-term hypertension. The long-term neurological effects of women with eclampsia have also been subjected to investigation. It has been found that women who have eclampsia or pre-eclampsia are at a greater risk of death from IHD than the women with normal pregnancies or women in the reference population (Jonsdottir et al, 1995). In a Norwegian cohort study women with pre-eclampsia who delivered pre-term were eight times more likely to die from IHD than women who had gestational hypertension and whose pregnancy went to term (Irgens et al, 2001).

1.1.6 The diagnosis of pre-eclampsia

Pre-eclampsia/eclampsia has its share of history and antiquity. The following short account is based on reviews by Irgens et al (2001). The pre-Hippocratic Kahun Papyrus from Egypt dating back almost 3000 years alluded to eclampsia. Ancient Greeks also recognized pre-eclampsia: "In pregnancy, the onset of drowsy headaches with heaviness is bad; such cases are perhaps liable to some sort of fits at the same time". There seems to be no mention of the condition, however, in any of the authenticated writings of Hippocrates, but Galen's commentaries on Hippocrates' aphorisms suggest that he knew of the violent manifestations in pregnancy. Similarly there is no mention of it in the writings of Susrutha the great Indian physician in the sixth century BC. In the tenth century AD, a famous monastic physician St. Gall had a trick played on him. It seems that he was about to examine the Duke of Bavaria

and, as a trick the worthy nobleman substituted for his urine that of a woman who was pregnant. The priest after making his examination made the solemn announcement that the Duke would give birth to a child. This incident is perhaps the earliest record of recognition of proteinuria in pregnancy, albeit somewhat indirect. Scientific writing on pre-eclampsia/eclampsia really begins in the seventeenth century. Mauriceau, Barton, and Blundell all recognized the symptoms of pre eclampsia. In 1840, Rayer observed proteinuria in three oedematous pregnant women. It was Lever and Simpson, however, who discovered proteinuria in eclampsia independently of each other in 1843. The hard bounding pulse of women with eclampsia had suggested arterial hypertension to the old time clinicians. Vinay, in 1884, used a primitive sphygmomanometer and found blood pressures ranging from 160 to 200 mmHg in pregnant proteinuric women; pressures up to 160 mmHg were said to be normal as estimated by his instrument. The discovery of eclamptic hypertension is generally credited to Vaquez and Nobecourt in 1897, but they remarked that they had confirmed Vinay's observation.

1.1.7 The differential diagnosis of pre-eclampsia

The appearance of hypertension and proteinuria for the first time in pregnancy clearly presents a diagnostic dilemma. It may be pre-eclampsia *per se*; it may be a long term problem antedating pregnancy which may or may not have been aggravated during pregnancy; rarely, it may be a previously undiagnosed medical problem such as phaeochromocytoma (Schenker and Chowers, 1971) by chance coinciding with pregnancy; or much more rarely it may be a pregnancy specific condition such as hydatidiform mole (Newman and Eddy, 1988) or Ballantyne syndrome presenting as pre-eclampsia.

1.1.8 Hypertension and pre-eclampsia

Hypertension is a *sine qua non* of pre-eclampsia. It is also a common medical problem independent of pregnancy. Women with chronic hypertension and/or renal

disease are three to seven times more likely to develop superimposed further hypertension and proteinuria in pregnancy (Chesley, 1980). In a population study of over a million people in America, 4.9% of women under the age of 40 years were hypertensive and among them the condition was previously undiagnosed in 39.7% (Stamler et al, 1976). In England and Wales the prevalence of treated hypertension in women under the age of 44 years was 2.38% (Ryan and Majeed, 1999). In a Sri Lankan study, 13% of 2047 women aged between 20 to 40 years had a diastolic blood pressure of >90mmHg (Mendis et al, 1988). Data on variation of the prevalence of hypertension over time is scarce. In a small study of blood pressure and other cardiovascular parameters in 806 adult women of Northern Sweden over the period 1986 to 1999, the prevalence of hypertension in those between the ages of 20 to 44 years ranged from 4.3% to 2.3% (Jansson et at, 2003). The number of women in this age group, however, is too small to draw broad conclusions from this data. Records of normal blood pressure during early pregnancy may mask chronic hypertension due to the effect of the normal physiological reduction in blood pressure in the first half of pregnancy (MacGillivray et al, 1969). Women with chronic hypertension therefore, may appear normotensive when they start antenatal care and then may show an increase in blood pressure in the third trimester resembling pre-eclampsia. Hypertension occurring before 20 weeks of pregnancy, however, is almost certainly chronic hypertension. It is usually essential hypertension with no identified underlying pathology (Sibai, 2002). Davey and MacGillivray (1988) recommended the use of the traditional cutoff of resolution of blood pressure within six weeks to confirm the diagnosis of pre-eclampsia. In more recent recommendations this period has been extended to three months (Brown et al, 2001). There is concern, however, as to whether insisting on postpartum blood pressure normalisation would misclassify women with pre-eclampsia who may have developed chronic hypertension secondary to irreversible vascular damage caused by the pre-eclamptic state. If this were so, women who develop pre-eclampsia early

in pregnancy, and are as a result more likely to have a prolonged illness with greater vascular damage; and women who have recurrent pre-eclampsia, resulting in repeated vascular damage, would be expected to take longer to normalise their blood pressure postpartum and/or develop chronic hypertension later in life. Several groups have reported such observations (Chesley et al, 1976; Ferrazzani et al, 1994). It is difficult to determine how much time is adequate for normalisation of blood pressure due to the scarcity of systematic data on blood pressure in the immediate postpartum period. It is most likely, however, that the earlier recommendation that blood pressure should resolve by six weeks postpartum to confirm the diagnosis was not appropriate (Ferrazzani et al, 1994). It is also likely that there will also be a subgroup that will still be 'unnecessarily' on antihypertensive therapy at this time.

Blood pressure measurement is routine in antenatal care, but it is necessary to ensure that a standardised method is practised in pregnancy (Brown et al, 2001). One area of controversy is the measurement of diastolic blood pressure (Higgins and de Swiet, 2001). The current ISSHP recommendation is to use Korotkoff phase V (K5) (sound disappearance) to record the diastolic blood 19 pressure, and to use Korotkoff phase IV (K4) (muffling) only when the sound does not disappear (Brown et al, 2001) because K5 is detected more reliably than K4 during pregnancy (Blank et al, 1994), because K5 reflects the true diastolic pressure in pregnancy than K4 (Brown et al, 1994), and because the change from K4 to K5 in hypertensive pregnant women does not alter the figures for the incidence of morbidity for the mother or the baby (Brown et al, 1998).

2.2 Proteinuria

De novo proteinuria is the second feature of the diagnosis of pre-eclampsia. A distinct renal lesion, glomerular endotheliosis, has been described and widely accepted as pathognomonic of pre-eclampsia (Spargo et al, 1959). It resolves postpartum. The severity of proteinuria in preeclampsia correlates positively with the

extent and the severity of the endothelial changes seen in the glomerulus (Fisher et al, 1981). Glomerular changes similar to that of pre-eclampsia, however, have also been reported in women with pregnancies complicated by hypertension alone. Moreover, in an ethically highly controversial study, control renal biopsies of normal pregnant women also show such changes, albeit of a milder degree (Strevens et al, 2003). These observations suggest that it is possible that the severe glomerular changes seen in preeclampsia may be one extreme of the adaptation process in pregnancy rather20 than an abnormal condition specific to pre-eclampsia. In one of the early studies, glomerular endotheliosis was present in renal biopsies of 70% of primiparous women and 14% of multiparous women with pre-eclampsia diagnosed on the basis of *de novo* hypertension and proteinuria (McCartney, 1964). In another study renal biopsies of 84% of primiparous women with pre-eclampsia and 24% of multiparous women had glomerular endotheliosis (Fisher et al, 1981). These observations together with the observations on the prognostic significance of parity to the development of hypertension later in life (Chesley et al, 1976); and the reported impact of parity on the clinical presentation and on fetal growth and development (Gleicher et al, 1986) are the basis for the widely accepted view that genetic studies of pre-eclampsia should be confined to primigravid women (Higgins and Swiet, 2001). However, women in their second pregnancy who have experienced an early first trimester abortion in first pregnancy could also be included in such studies as their uterine morphology essentially resembles that of primigravid women. In fact the risk of developing pre-eclampsia in nulliparous women with a previous abortion who conceive again with the same partner is half that of nulliparous women who do not have such a history (Saftlas et al, 2003).

Renal protein excretion increases in normal pregnancy, and may reach levels of up to 300mg day in the third trimester of a normal pregnancy (Davison, 1985; Higby et al, 1994). Proteinuria in excess of 300mg/day is extremely rare in uncomplicated

pregnancies (Higby et al, 1994) and is usually associated with pre-eclampsia or renal disease. Rapid and reliable detection of significant proteinuria has always posed a challenge in clinical settings. Quantitative tests for proteinuria, although available in routine clinical practice in the West, are not widely available even in the best centres iof the developing countries. The cut-off point for detecting significant proteinuria with the HCT, however, seems not to have been formally established.

Pre-eclampsia is the most common cause of gross proteinuria in pregnancy. Other renal diseases that may give rise to a similar clinical picture, the diagnosis of which rely on a renal biopsy, include proliferative or membranoproliferative glomerulonephritis, minimal change nephrosis, lupus nephropathy, hereditary nephritis, diabetic nephropathy, renal vein thrombosis, and amyloidosis (Davison and Baylis, 1998). Non-glomerular renal disease such as reflux nephropathy and polycystic kidney disease can also give rise to significant proteinuria (Ihle et al, 1987). A renal biopsy, however, is almost never indicated to resolve the differential diagnosis of *de novo* proteinuria in pregnancy (Lindheimer and Davison, 1987).

2.3 Some pathophysiological aspects of pre-eclampsia

Pre-eclampsia has been termed the 'disease of theories' due to the confusion surrounding its aetiology (Ness and Roberts, 1996; Dekker and Sibai, 1998). It is possible that the hypertension and proteinuria that are relied on to define the condition may simply be the final common pathway through which more than one type of pathology is being expressed. Pre-eclampsia is now widely accepted to be a two-stage disorder: reduced placental perfusion usually secondary to abnormal placentation and a consequent maternal disorder characterized by endothelial dysfunction and a systemic maternal disease (Roberts and Redman, 1993; Roberts, 2000).

Stage 1: Reduced placental perfusion

Pre-eclampsia only occurs in the presence of a placenta and its resolution begins with the removal of the placenta. More than 70 years ago Page proposed that decreased placental perfusion results in pre-eclampsia. This has subsequently been confirmed by direct measurements of intervillous blood flow (Kaar et al, 1980) and by animal experiments where it has been shown that reducing blood flow to the pregnant uterus and placenta results in a condition remarkably similar to pre-eclampsia, including glomerular endotheliosis (Aladjem et al, 1983). In many cases reduced perfusion is secondary to abnormal placentation. In addition conditions that are associated with microvascular disease such as chronic hypertension and diabetes (Garner et al, 1990; Lao and Tam, 2001; Xiong et al, 2001; Bryson et al, 2003; Ostlund et al, 2004); and conditions that are thrombophilic such as anticardiolipin antibody syndrome (Branch et al, 1989) may also decrease blood supply to the placenta and increase the pre-eclampsia risk. Moreover, obstetric conditions that increase placental mass such as multiple pregnancies (Coonrod et al, 1995) and hydatidiform mole (Newman et a, 1988) can have the same effect, possibly due to a 'relative' decrease of placental blood flow.

2.4 Placentation: Morphological changes

The process of human placentation is an invasive phenomenon in which embryo-derived cytotrophoblastic cells progressively integrate into maternal tissue. After implantation specialized epithelial cells of the placenta that attach the fetus to the mother- cytotrophoblasts, differentiate along one of two pathways. In the first, cytotrophoblasts fuse to form the multinucleate syncytium that covers the floating chorionic villi. These villi, which are in 42 direct contacts with maternal blood in the intervillous space, perform nutrient and gas exchange functions for the fetus. In the second pathway, extra villous cytotrophoblasts in the anchoring villi proliferate to form a shell lining the uterine cavity, and invade the uterine wall (interstitial invasion)

and its blood vessels (endovascular invasion). Cytotrophoblasts that invade the uterine wall (interstitial cytotrophoblasts) reach the superficial myometrium by the eighth week of gestation (Pijnenborg et al, 1981). Later, they invade deeper into the myometrium especially at the centre of the placental bed (Pijnenborg et al, 1983). When they reach the end of their invasion path they fuse to form multinuclear giant cells (Pijnenborg et al, 1996). Endovascular cytotrophoblasts invade the spiral arteries and migrate along the arteries to reach myometrial spiral arteries by the tenth week of gestation. This process, however, may continue for several more weeks (Pijnenborg et al, 1983). It has been hypothesised that endovascular invasion occurs in two waves – a first wave at the beginning of pregnancy following fecundation and a second deeper wave at the end of the first trimester around 14 to 16 weeks of gestation that involves the inner third of the myometrium (Pijnenborg et al, 1996). There is also data, however, that suggest a progressive endovascular migration into myometrial arteries rather than it occurring in two stages (Robson et al, 2001).

The process of trophoblast invasion, in addition to physically anchoring the fetal placenta to the uterus, transforms the small diameter muscular spiral arteries into large-diameter low resistance flaccid vessels that carry blood to the intervillous space (Pijnenborg *et al.*, 1983). It is clear that this process, named physiological change, has to take place in both decidual and myometrial segments of spiral arteries as an essential prerequisite for the provision of an adequate blood supply to the rapidly growing fetus and placenta. The changes in myometrial arteries never return to a 'non-pregnant'state, so that second and subsequent pregnancies begin with an advantage in terms of flow (Brosens et al, 1967; Khong et al, 2003). Interstitial trophoblast invasion is deficient in pre-eclampsia, but endovascular invasion is greatly impaired and physiological changes do not take place in both decidual and myometrial spiral arteries. They remain low calibre high resistance vessels that are

unable to provide an adequate blood supply to the intervillous space (Khong et al, 1986).

2.5 Preeclamsia, Eclampsia and Serum Mineral

Malnutrition is a common problem in our country. Pregnancy imposes a great stress on the nutritional reserves. There is depletion of essential nutrients like Vitamin- B complexes, Vitamin-A, Folic acid, Iron and Calcium. Intake of this essential nutrient in poor income women is far below than recommended daily allowances. Decreased protein intakes causes decrease calcium absorption from the gut and stimulate parathyroid hormone secretion. Thereby enhance calcium reabsorption from bone and maintain calcium balance (Joel, 1994). There is progressive decline in magnesium consumption from 475-500mg/day in 1900—1908 to 175-225mg/day in 1990- 2002 due to widespread consumption of processed foods and decreased consumption of fresh foods. Nuts and green leafy vegetables are good sources of magnesium. Eclampsia is primarily a convulsive state, despite extensive physiological, biochemical and anatomical changes that occur during pregnancy and may be local or systemic (William, 1994), serum electrolytes and ionized Ca^{2+} level remain normal (Rashid, 2009). Magnesium is one of four cations that must be kept in balance in extra cellular fluid to regulate all body functions that require ATP (Michael, 2001; Alan, 1988). In women with eclampsia, magnesium deficiency is suspected. Because during normal pregnancy plasma volume increases 50%, increased metabolic and endocrine activity of mother and fetus, estrogen and progesterone levels which increases as pregnancy advances (William, 1994; Dutta, 2005), elevates the body's demand for magnesium and more and more magnesium are utilized and produce physiological hypomagnesaemia and symptoms of hypomagnesaemia (Joel, 1994). On the other hand, if pregnancy remain uncared, women have no ANC, decreased dietary supply of protein, vitamins and minerals preexisting protein energy malnutrition, low maternal age, low income, lower quality

of life, these lead to severe micronutrient deficiency particularly magnesium deficiency that causes tissue hyperirritability and convulsion (Joel, 1994). Serum magnesium level is tightly regulated in a narrow range of approximately 0.7 to 1.0mmol/l because only 1% of the total body content of magnesium is extra cellular (Michael, 2001). When serum ionized magnesium level falls below 0.7 m mol/L are indicative of magnesium deficiency. But patient remain asymptomatic (Michael, 2001). Symptoms appear when level falls below 0.5mmol/L. This level indicate depleted total body magnesium stores because magnesium reside in side the cell, total body magnesium deficit is often ~200mmol (4800mg) by the time serum level falls to <0.5mmol/L (Michael, 2001). Magnesium is a natural calcium blocker. Alteration of intra and extra cellular magnesium concentration may affect cell function through their effect on calcium handling. There is evidence that magnesium acts by opposing calcium dependent arterial vasoconstriction by antagonizing the increase in intracellular Ca^{+2} concentration caused by ischemia. Magnesium depletion causes impaired parathyroid hormone secretion and function (Michael, 2001) and chronic magnesium deficiency causes damage to the cardio vascular, renal and neuromuscular tissue (Alan, 1988). It enhance the contractility of several vascular beds in vitro, including coronary arteries, the mesenteric vasculature and umbilical vessels and may potentiate the contractile response to humoral agents such as nor adrenalin, prostaglandins and angiotensin II and leads to wide spread vasoconstriction (Maurice, 1994). Vasoconstriction causes increased resistance to blood flow and accounts for the development of arterial hypertension. Vasoconstriction induced ischemia may be sufficient enough to lower the activity in affected region of brain.

2.6 Calcium, magnesium and zinc in Pre-Eclampsia

Jain et al (2009) concluded in their study that pre-eclampsia is the most common medical complication of pregnancy associated with increased maternal and infant

mortality and morbidity. Its exact etiology is not known, although several evidences indicate that various elements might play an important role in pre-eclampsia. This study was carried out to analyze and to compare the concentration of calcium, magnesium, and zinc in the serum of women with pre-eclampsia and in normal pregnant women. Fifty clinically diagnosed patients with pre-eclampsia (25 with mild and 25 with severe pre-eclampsia) and 50 normal pregnant controls were enrolled in this study. The serum calcium, magnesium, and zinc levels were estimated with an atomic absorption spectrophotometer. The mean serum levels of calcium, magnesium, and zinc in normal pregnant group were 2.45±0.18 mmol/L, 0.79±0.13 mmol/L, and 15.64±2.4 µmol/L, respectively, while in mild pre-eclamptic group, these were 2.12±0.15 mmol/L, 0.67±0.14 mmol/L, and 12.72±1.7 µmol/L, respectively. Serum levels in severe pre-eclamptic group were 1.94±0.09 mmol/L, 0.62±0.11 mmol/L, and 12.04±1.4 µmol/L, respectively. These results indicate that reduction in serum levels of calcium, magnesium, and zinc during pregnancy might be possible contributors in etiology of pre-eclampsia, and supplementation of these elements to diet may be of value to prevent pre-eclampsia.

2.7 Serum calcium and magnesium in pre-eclamptic and normal pregnancy

Preeclampsia is a common gestational disorder which complicates 5-8% of pregnancies and it is associated with maternal, fetal and neonatal morbidity and mortality. Alterations in serum calcium (Ca) and magnesium (Mg) levels have been suggested as effective factors in causing preeclampsia. A study was conducted to compare serum calcium and magnesium levels in preeclamptic and normal pregnant women. In this case-control study, 50 preeclamptic and 50 normal pregnant women referring to Ghaem Hospital, affiliated to Mashad University of Medical Sciences, were selected during 2005. Blood samples of both groups with similar gestational ages were collected and compared for calcium and magnesium concentrations.

Serum magnesium levels in the preeclamptic women were significantly lower than those of individuals with normal pregnancy.

2.8 Iron, copper and zinc levels in pre-eclampsia

Upadhyaya et al (2004) conducted a study in India and demonstrated that pregnancy is associated with increased demand of all the nutrients like Iron, Copper, Zinc etc. and efficiency of any of these could affect pregnancy, delivery and out come of pregnancy. With this consideration, the study was conducted on 80 mothers and newborns and 20 age matched control women. Out of 80 mothers, 34 had Iron deficiency anemia and their Hb levels were below 9.0 gm/dl. Pregnant women had significantly lower Iron and Zinc levels while Copper and Total Iron Binding Capacity (TIBC) were significantly higher ($P<0.001$). Newborns had significantly elevated Iron and Zinc levels and low levels of Copper and TIBC as compared to their mothers irrespective of Iron deficiency anemia. Micronutrient status of newborn was found to be dependent on their mother's micronutrient status. Besides, results also suggest micronutrient interactions, which are reflected in Iron/Zinc, Iron/Copper and Zinc/Copper ratios. In view of this, there is need for proper, adequate and balanced micronutrient supplementation during pregnancy to affect a healthy outcome. In Bangladesh R Akter, M Rashid did a research work on serum magnesium and eclampsia subjects aimed to determine the level of serum ionized magnesium in eclampsia patient before and 24 hours after giving the loading dose of magnesium sulfate and if low and also assess the relationship between levels of ionized magnesium in serum and occurrence of eclamptic convulsions. The author concluded from the study results that low level of ionized magnesium in serum may be the cause of eclamptic convulsion. The present study aimed to assess the association of preeclampsia and eclampsia with serum calcium, magnesium, copper, iron, zinc.

CHAPTER 3
SUBJECTS AND METHODS

3.1 Study population

This research was a case control study conducted on 33 pre-eclampsia & 44 eclampsia case subjects. Twenty seven normotensive pregnants were taken as pregnant control and 29 normotensive non pregnant women were also recruited to know the basal mineral status. The pre-eclamptics, eclamptics and pregnant cases were recruited from Salimullah Medical College Hospital, Dhaka Medical College Hospital and Bangabandhu Sheikh Mujib Medical University. Non-pregnant control subjects were selected from the patient's attendants. The study was carried out during January 2008 to December 2009.

Required permission was taken from the departments concerned for recruitment of patients. All patients included in this study were informed about the nature of the study and agreed to participate voluntarily in this study.

Inclusion Criteria

- Pre-eclampsia subjects were included within 28 to 42 weeks of singleton gestation
- Eclampsia considered according to WHO criteria.
- Mild preeclampsia is defined as a blood pressure of at least 140/90 mmHg measured on two occasions each 6 hours apart, accompanied by proteinuria of at least 300 mg per 24 hours.
- Eclampsia is defined as having one or more of the following criteria: blood pressure of at least 160/110 mmHg measured on two occasions each 6 hours apart, proteinuria of at least 5 g per 24 hours, or at least 3+ on dipstick testing, oliguria of lesser than 500 ml per 24 hours, cerebral or visual disturbances, pulmonary edema or cyanosis, epigastric or right upper quadrant pain, impaired liver function, thrombocytopenia, fetal growth restriction.
- Fetal growth restriction is defined as the condition in which the newborn has a birth weight lesser than 10% for gestational age.
- Age- and socioeconomic-matched healthy normotensive of 28 to 42 weeks of singleton gestation with nil urinary protein will be recruited by convenience as the controls for both the pre-eclampsia and eclampsia subjects.

Exclusion criteria

- Includes history of hypertension and proteinuria before conception or before 20 weeks of gestation, any associated-medical disorders, history'-of any mineral during last one year.
- Patients with history of onset of convulsion before 48 hours also excluded from the study.

Inclusion criteria for control subject

- 28 to 42 wks of singleton gestation (pregnant control) and non-pregnant women of child bearing age (non-pregnant control).
- normal BP throughout pregnancy.
- urinary protein nil.

Exclusion criteria for the control group

- Any associated medical disorder
- History of taking antioxidant vitamin supplementation during last 9 months.

3.2 Plan for data collection

History was taken, clinical examination and relevant biochemical tests were carried out. Information was recorded in a pre-designed data sheet.

3.3 Estimation of urinary protein, glucose and pH

About 10 ml of mid stream specimen of random urine was collected in a clean and dry test tube. Urinary protein and sugar and pH were detected by reagent strips (Dipstick-Boehringer Mannheim). The test strip was dipped into the urine up to the mark for no more than one second. The strip then removed and wiped off excess urine. The strip was held in a horizontal position to prevent mixing of chemicals from adjacent reagent areas and / or contaminating the hands with urine. The strip was properly oriented near the appropriate colour chart on the container label. Protein changed the colour of Tetrabromphenol blue. A positive reaction was indicated by a colour change to yellow/green. Urinary protein 2+ or more (100mg/dl) was considered as positive. If sugar or pH>6 was detected in urine, the woman was excluded from the study.

3.4 Collection of blood samples

Five ml of venous blood was collected from each of the cases of pre-eclampsia and eclampsia patients as well as from the control subjects (normal pregnant and non-pregnant), with a disposable syringe, by peripheral venipuncture, taking full aseptic precaution. Blood was drawn for single time from each of the subject. From the cases and the control pregnant women, blood samples were collected during their antenatal period. In cases of eclamptic patients, blood was taken soon after admission. After removal of the needle from the syringe, blood samples were collected in screw capped 5ml vials, wrapped with aluminum foil. Serum was extracted within 1h of collection and stored in dark at–20°C until analysis of minerals.

3.5 Analysis of serum mineral

Serum calcium, magnesium, copper, zinc and iron were measured by atomic absorption at Centre for Excellence, University of Dhaka.

The measurements of minerals were performed with a Shimadzu 680 Atomic Absorption Spectrometer equipped with a hollow cathode lamp and a deuterium background corrector, at respective wavelengths- 422.7nm for Ca, 324.8nm for copper, 248.3nm for iron, 213.9nm for zinc; using an air-acetylene flame. Series of working standard solutions of Ca, Mg, Cu, Zn and Fe were prepared from the stock standard solutions and appropriate dilutions of the serum samples were done for better absorbance (Hossain et al, 2007; Skoog *et al*, 2006). Standard curves were made for each of the minerals and with the help of that curves the concentration of the serum samples were calculated.

3.6 Statistical Analysis

The SPSS (12.5 version) software package was used for statistical analysis. Data were presented as mean ± SD and median range. Comparison of serum levels of the elements between the groups was performed by Student's *t* test, ANOVA. Association was calculate using regression curve and corelation co-efficient and $P<0.05$ was considered as statistically significant.

CHAPTER 4
RESULTS

4.1 Serum mineral level

Serum mineral levels in pre-eclampsia, eclampsia, pregnant control and non-pregnant women are described in table 1. Except iron value, there was significant ($p<0.05$) increase in serum concentrations of Ca and Mg in eclampsia as compared to those in pre-eclampsis, but concentrations of Cu, and Zn in pre-eclamsia were higher than those in eclampsia. Although, a significant variation was noted in the mineral content between pre-eclampsia and eclampsia, the change in mineral value between pre-eclampsia and pregnant control was not significant. However, serum Cu and Zn levels in pre-eclampsia were significantly higher ($p<0.05$) than the non-pregnant women. Calcium and magnesium concentrations in eclampsia were found significantly ($P<0.05$) high as compared to those in the pregnant control,but in case of copper it is reverse. Compared to non-pregnant women, all of the serum mineral levels were found high in eclampsia. It was further observed that except calcium, all of the minerals in pregnant women were higher than those in the non-pregnants.

It was also noted that mineral values among the study women were found to be within the normal range (Young, 1998). However, the serum magnesium level is in the lowest limit.

Table 1: Comparison of serum calcium, magnesium, copper, zinc and iron level among pre-eclampsia, eclampsia, pregnant control and non-pregnant women

Mineral level* mmol/L	Pre-eclampsia[a] (n=44)	Eclampsia[b] (n=33)	Pregnant control[c] (n=27)	non-pregnant women[d] (n=29)
Calcium[1]	2.36±0.221	2.47±0.291	2.32±0.244	2.28±0.190
Magnesium[2]	0.817±0.489	0.847±0.054	0.815±0.069	0.811±0.071
Copper[3]	0.017±0.002	0.015±0.002	0.016±0.002	0.013±0.001
Zinc[4]	0.016±0.002	0.014±0.002	0.015±0.002	0.014±0.001
Iron[5]	0.019± 0.004	0.021±0.008	0.023± 0.002	0.017± 0.001

*values were expressed in mean±sd

Level of significance ($p<0.05$) was expressed by *student t test*.

1ab t=1.87, p=0.06	1ac: t=0.65, p=0.51	1ad t=1.54, p=0.12	1bc t=2.18, p=0.03	1bd t=0.019,p=0.003	1cd t=0.69, p=0.490
2ab t=2.52, p=0.014	2ac: t=0.15, p=0.88	2ad t=0.39, p=0.693	2bc t=1.96, p=0.055	2bd t=2.238,p=0.007	2cd t=5.54, p=0.000
3ab t=3.39, p=0.001	3ac: t=1.24, p=0.218	3ad t=7.28, p=0.000	3bc t=2.06, p=0.044	3bd t=2.80, p=0.007	3cd t=5.55, p=0.000
4ab t=2.06, p=0.043	4ac: t=0.56, p=0.580	4ad t=5.68, p=0.000	4bc t=1.20, p=0.236	4bd t=3.34, p=0.001	4cd t=4.03, p=0.000
5ab t=0.33, p=0.744	5ac: t=0.40, p=0.694	5ad t=0.009, p=0.993	5bc t=0.009, p=0.993	5bd t=4.52, p=0.000	5cd t=5.93, p=0.000

4.2 Comparison of serum mineral among case and control subjects

One way analysis of variance was performed to test relation to and significance of serum mineral levels among the pre-eclampsia, eclampsia, and pregnant control (table 2). It was indicated that there had significant ($p<0.05$) relationship to calcium, magnesium and copper levels of the case and control subjects.

Table 2: Comparison of serum calcium, magnesium, copper, zinc and iron level among pre-eclampsia, eclampsia, and pregnant control

Mineral level* mmol/L	Pre-eclampsia (n=44)	Eclampsia (n=33)	Pregnant control (n=27)	ANOVA Level of significance (p<0.05
Calcium	2.36±0.221	2.47±0.291	2.32±0.244	0.05
Magnesium	0.817±0.489	0.847±0.054	0.815±0.069	0.04
Copper	0.017±0.002	0.015±0.002	0.016±0.002	0.003
Zinc	0.016±0.002	0.014±0.002	0.015±0.002	0.14
Iron	0.020± 0.004	0.020±0.004	0.020± 0.002	0.92

*expressed as mean±sd.
ANOVA was performed as a test of significance.

When ANOVA was done to assess the relationship of serum minerals among the pre-eclampsia, eclampsia, and non-pregnant; it was observed to have significant ($p<0.05$) relationship to all of the tested minerals of the case and non-pregnant subjects (table 3).

Table 3: Comparison of serum calcium, magnesium, copper, zinc and iron level among pre-eclampsia, eclampsia, and non-pregnant control

Mineral level* mmol/L	Pre-eclampsia (n=44)	Eclampsia (n=33)	non-Pregnant (n=29)	ANOVA Level of significance (p<0.05)
Calcium	2.36±0.221	2.47±0.291	2.28±0.190	0.008
Magnesium	0.817±0.489	0.847±0.054	0.811±0.071	0.03
Copper	0.017±0.002	0.015±0.002	0.013±0.001	0.000
Zinc	0.016±0.002	0.014±0.002	0.014±0.001	0.000
Iron	0.020± 0.004	0.020±0.004	0.017± 0.001	0.000

*expressed as mean±sd.
ANOVA was performed as a test of significance.

4.3 Ratio of mineral in the case and control subjects

Significance of ratio of copper to iron, magnesium to calcium and zinc to copper were calculated for the case and control and baseline subjects (figure 1, 2, 3). It was shown that only copper to iron ratio in eclampsia was found significantly high as compared to that in other subjects. No other ratios of the minerals were observed to be significant in any of the subjects.

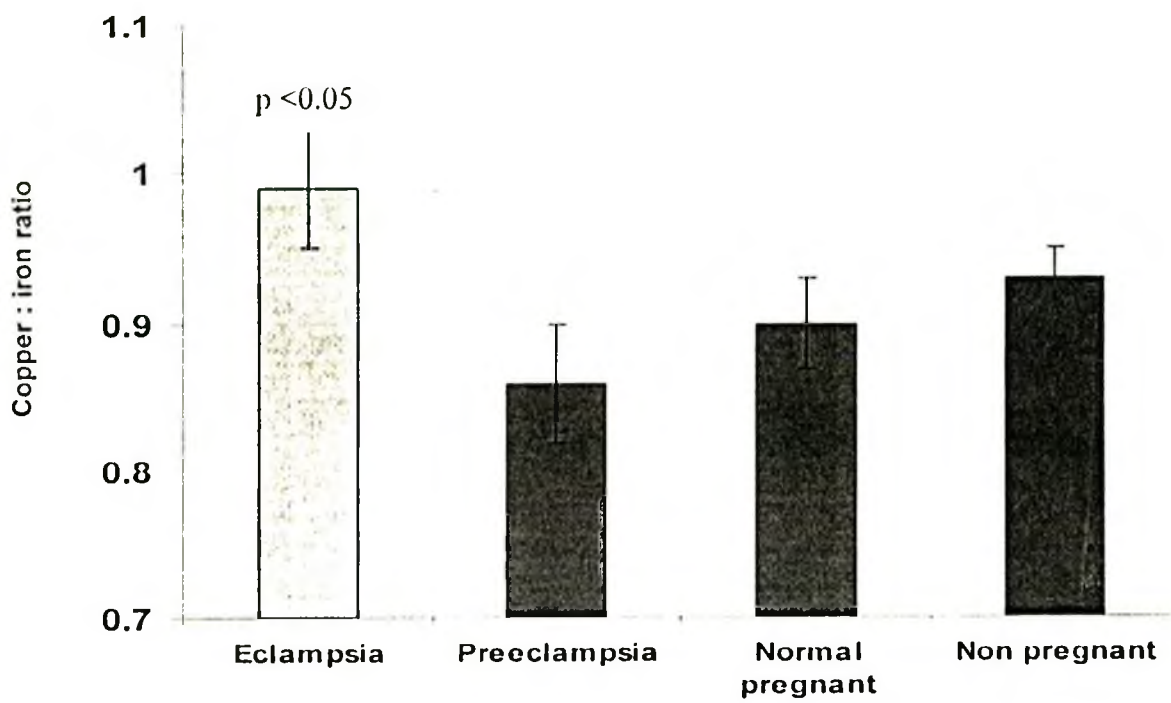

Figure 1: Ratio of copper to iron in different subjects

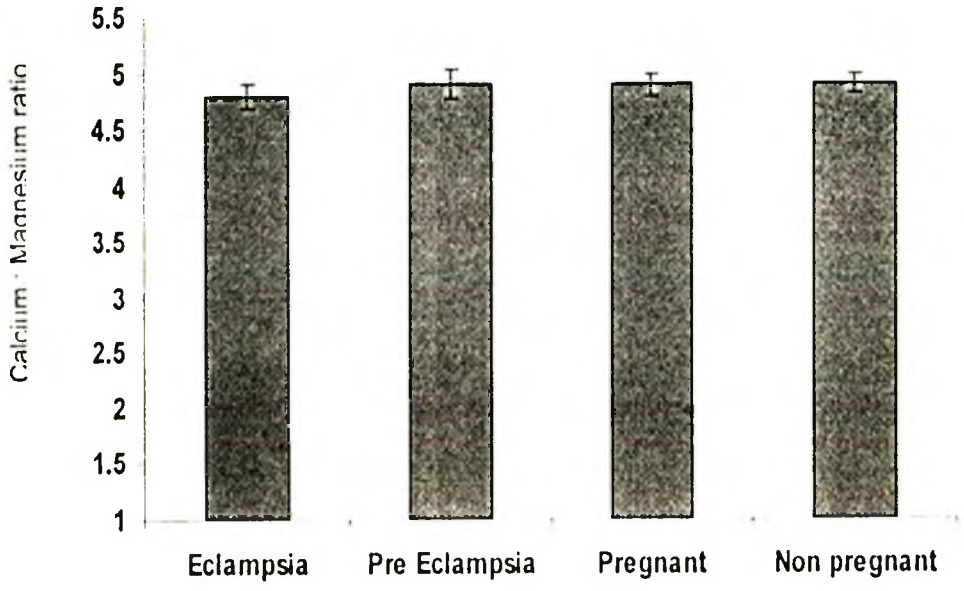

Figure 2: Calcium : Magnesium ratio

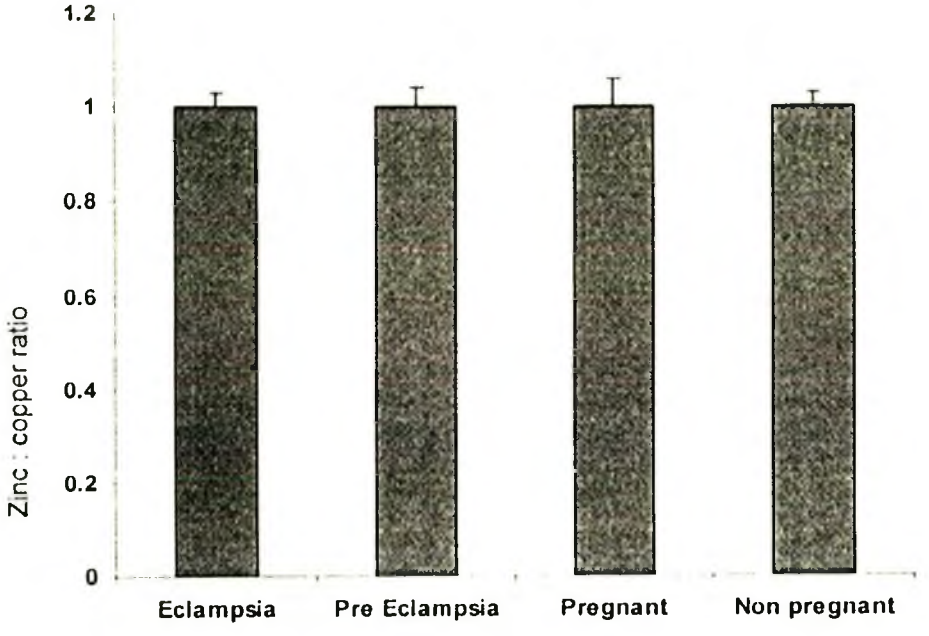

Figure 3: Zinc : copper ratio

4.4 Correlation between magnesium and zinc, and magnesium and iron

When Pearson correlation were performed to find out the association between magnesium and zinc in eclampsia, the result showed magnesium was positively (r=0.35, p=0.06) associated with serum zinc but the association was not strongly significant (figure 4). There was no association between magnesium and zinc in pre-eclamsia Magnesium was noted to be positively associated with serum iron in both cclamsia and pre-eclamsia subjects (figure 5).

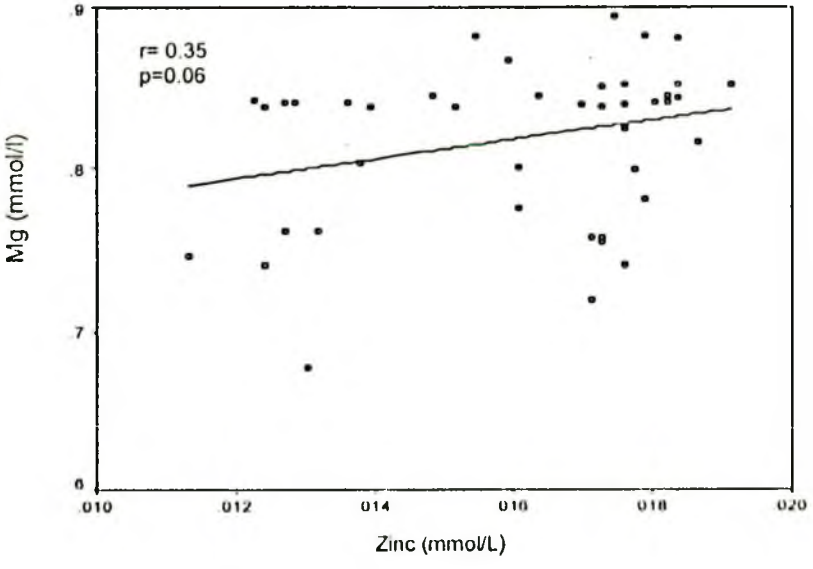

Figure 4: Relationship between magnesium and zinc in eclampsia

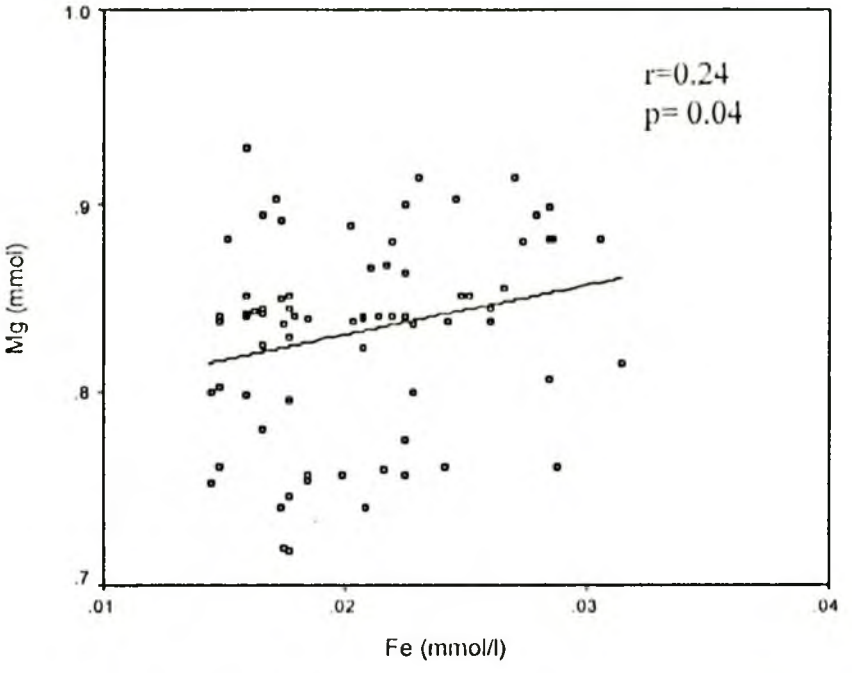

Figure 5: Relationship between Mg and Fe in pre-eclampsia and eclamsia subjects

CHAPTER 5
DISCUSSION

Pre-eclampsia and eclampsia are pregnancy disorder with worldwide significance to mothers and infants. Its greatest impact is in developing countries, where it accounts for 20-80% of the strikingly increased maternal mortality. However, even in developed countries there is a major effect, primarily on the foetus. It is a systemic disease of late pregnancy that affects about 5 - 7% of all pregnancies and is the most common. Yet it is least understood disorder of pregnancy (Ziael et al, 2008; Lou et al, 2008). Despite numerous studies, the etiology of preeclampsia has not yet been fully elucidated. Some studies have shown that changes in the levels of blood trace elements in pre-eclamptic patients may implicate its pathogenesis (Bringman et al, 2006; James et al, 2006) while others have failed to show an association of blood levels of trace elements and prevalence of pre-eclampsia (Caughey et al, 2005). Oxidative stress also claims to play a significant role in the pathogenesis of preeclampsia (Hubel, 1999; Roberts and Hubel, 1999).

Currently immunologic maladaption has been proposed as a pathogenic mechanism for preeclampsia. Defective regulation of the complement system induces this complication. Pregnancy in women with systemic lupus erythematosus (SLE) or antiphospholipid antibodies (APL Ab)—autoimmune conditions characterized by complement-mediated injury—is associated with increased risk of preeclampsia and miscarriage (Salmon et al, 2011). Mutations in membrane cofactor protein (MCP), complement factor I (CFI), and complement factor H (CFH) appear to predispose to the development of disease (Caprioli et al, 2006).

5.1 Serum mineral level

Blood calcium and magnesium have a relaxant effect on the blood vessels of pregnant women (Skjaerven et al, 2002). Serum magnesium may have significant effects on cardiac excitability and on vascular tone, contractility and reactivity. Low serum concentration of calcium and/or magnesium led to constriction of smooth

muscles in blood vessels and increase of vascular resistance, and thus increase blood pressure (Power et al, 1999; Lopez, 2000).

In the present study a significant ($p<0.05$) increase of serum Ca and Mg in clampsia as compared to those in pre-eclampsis, but compared to pregnant control it was insignificant as reported by Magri et al (2003). Concentrations of Cu, and Zn in pre-eclamsia were higher than those in eclampsia. There was significant increase in serum Ca and Mg, but decrease in Cu in the eclampsia as compared to those in pregnant control. Some studies showed that serum magnesium was even higher in the pre-eclamptic patient than that in pregnant (Sanders et al, 1999; Seydoux et al, 1992). It is to be further noted here that mineral values among the study women were found to be within the normal range and serum magnesium level is in the lowest limit (Young, 1998).

Serum copper, zinc, calcium and magnesium levels have been compared between pre-eclamptic and healthy pregnant women in various studies. Some results showed that copper, zinc, and calcium levels were significantly lower in pre-eclamptic patient, whereas magnesium concentrations showed no significant differences between the two groups (Kumru et al, 2003). This study showed that change in serum mineral levels in pre-eclampsia and eclampsia may have implication on the pathogenesis of this pregnancy complications.

5.2 Ratio of mineral in the implication of disorders

Result showed that only copper to iron ratio in eclampsia was significantly high. Ratios of other minerals did not show any significant change. It could be concluded that high copper to iron ratio in eclampsia may have association with the complications.

5.3 Relationship between serum mineral and complications

Pearson correlation showed magnesium was positively associated with serum zinc in eclampsia. Magnesium was noted to be positively associated with serum iron in both cclamsia and pre-eclamsia subjects. This correlation may contribute to the pregnancy complications. However, some observation did not find a relationship between the serum mineral levels and gestational hypertension (Mari et al, 2003).

Conclusion

This study revealed that there have significant variations in serum mineral values between pre-eclampsia and eclampsia and with the pregnant control. Serum copper to iron ratio in eclampsia was indicated significantly high. A significant positive correlation was observed in serum magnesium change with zinc in eclampsia and magnesium change with iron in both the pre-eclampsia and eclampsia. Therefore, it may be concluded that change in mineral value may in pre-eclampsia and eclampsia may have implication on the pathogenesis of this pregnancy complications. Uncovering the aetiology of this pregnancy complications would help prevention and management of this pregnancy related complication, and thus would support the health care facilities

Limitation

- Cross sectional study needs a large sample size.
- Hemoglobin, ferritin and transferrin need to be analyzed.

Recommendation

Change in serum mineral concentration like calcium and magnesium can lead to the alteration of blood pressure. Magnesium is an essential cofactor for many enzyme systems and also plays an important role in neurochemical transmission and peripheral vasodilatation. Variation plasma and intracellular calcium can induce elevation of blood pressure in gestational hypertension- pre-eclampsia. Therefore, to comment on the influence of mineral in the pathogenesis of gestational hypertension, in addition to analyzing the serum mineral content, intracellular mineral concentrations are necessitated to be investigated.

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Appendix

DATA COLLECTION FORM

Subject Identification :

Code no-
Reg no
Date of admission-
Ward-
Bed-
Particulars of the subject
Name:
Husband's Name:
Address:
Age in yesrs
Economic status:

History of present pregnancy-

Para
Gravida-
Gestational age in wks
antenatal check up
1. Yes 2. No
Onset of hypertension (in weeks)
Onset of proteinuria (in wooks)
Onset of convulsion (in hours)
Physical examination
Oedema- 1. mild 2. moderate 3. Severe

Blood pressure in mmHg

SBP after ≥ 6 hr-
DBP after ≥ 6hr-

Height of the uterus(in cm)

FHS-

regular
irregular

Investigation:

Urine :
protein-
sugar

Anaemia

Nill
Mild
Moderate
Severe

Oedema

Nill
Mild
Moderate
Severe