SERUM LEVELS OF SELECT MICRONUTRIENTS IN PRIMIGRAVIDA WITH PREECLAMPSIA VERSUS NORMOTENSIVE COUNTERPARTS: A CASE-CONTROL STUDY AT THE KENYATTA NATIONAL HOSPITAL

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DECLARATION

I hereby confirm that this thesis represents my original work and has not been presented elsewhere.

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DEDICATION

In loving memory of:

Mr. Francis Pulei Ole Munkush, my doting father, you truly were an inspiration. Thank you so much for setting such high goals for me. Baba, you did everything to make me work harder, including incentives and tough love! I will uphold the diplomacy and dedication you nurtured in me. You also taught me to handle every individual differently as you did your children. I will always miss you.

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ABBREVIATIONS AND SYMBOLS

PE	Preeclampsia
Ca	Calcium
Se	Selenium
Zn	Zinc
Mg	Magnesium
WHO	World Health Organization
RCOG	Royal College of Obstetricians and Gynaecologists
ACOG	American College of Obstetricians and Gynaecologists
SBP	Systolic Blood Pressure
DBP	Diastolic Blood Pressure
LMIC	Low and middle – Income countries

ABSTRACT

Background: Preeclampsia is a multisystem disorder of unknown etiology that is unique to pregnancy. It remains to be one of the leading causes of maternal mortality as well as morbidity and long-term disability to mothers and newborns in sub-Saharan Africa. The status of serum levels of calcium, zinc and selenium as well as vitamin D has been implicated in the pathogenesis of preeclampsia. Indeed calcium supplementation is now recommended for its prevention in low-income settings. However, the supplementation of the other micronutrients remains debatable. This study looked into serum quantities of calcium, selenium, zinc and Vitamin D in women with preeclampsia compared to that of normotensive women in our setup. The findings of this study help to acknowledge these mineral deficiencies as part of the risk factors for preeclampsia in our population and subsequently recommend their supplementation as a preventive measure and encourage dietary changes.

Broad Objective: To investigate the serum levels of select micronutrients (Vitamin D, calcium, zinc, and selenium) in preeclamptic and normotensive primigravid women

Methods:

Study design: This was a case-control study in which 54 primigravida with preeclampsia (cases) were compared with 54 primigravida who were normotensive (controls) and were matched for gestation and age.

Study setting: Kenyatta National Hospital is a regional Teaching and Referral hospital with 1800 bed capacity. It has a busy reproductive health department that conducts 20-50 deliveries per day. The prevalence of preeclampsia in this institution is 5%.

Study population: A hundred and eight primigravid women comprised the study population. These included primigravida with preeclampsia ≥ 20 weeks gestation who were compared with their normotensive counterparts matched for gestation and age. These were 54 cases and 54 controls matched for age at ±2 years and gestational age of ± 2 weeks.

Data collection procedures: After approval from the Kenyatta National Hospital/University of Nairobi-Ethics/Review Committee (KNH/UON-ERC) and seeking permission from the hospital administration, primigravida with preeclampsia, cases, as well as the normotensive controls were consented. They were then interviewed and had their demographic, obstetric parameters and dietary habits recorded in data collection sheets and blood samples obtained from them for determination of serum calcium, selenium, zinc and Vitamin D levels. The blood taken was put in specimen bottles with a serum separator gel and taken to a diagnostic laboratory in Nairobi for analysis.

Data management and analysis: Data on demographic, obstetric and biochemical parameters, consumption of specific nutrient rich foods was coded entered into SPSS version 20. Means of the biochemical parameters were obtained for the two groups and compared using the Students T test, a P value of <0.05 was considered statistically significant. Chi square and the Fisher's exact test were used for categorical data.

Results: A hundred and eight participants were included in this study (54 cases and 54 controls). Seventy-two percent of these women were between ages 20-30 years. Most, 90% had attained at least secondary education with an almost half and half distribution between the cases and the controls. Sixty-one percent of the study population had an average income of less that Ksh 10,000. The average gestational age was 35.2 ±4.4 weeks for the cases and 35.4±4.4 weeks for the controls. For the cases, 38.9% had preeclampsia without features of

severity (formerly mild preeclampsia) while 61.1% had severe preeclampsia. The average systolic and diastolic blood pressure among the cases was 155.7±17.4 and 105 ±10.2 respectively. Vitamin D deficiency was recognized in 31% of the entire study population. The distribution of these women with Vitamin D deficiency was such that 50% of those with preeclampsia were deficient compared to 27% normotensive women ($p<0.001$). The mean serum vitamin D level amongst cases and controls was 20.8±10.2 ng/ml and 28.6±7.9 ng/ml respectively ($p<0.001$). Serum calcium levels were 2.2±0.3mmol/l for the cases and 2.3±0.09mmol/l for the controls ($p=0.024$). We did not find statistically significant association between Selenium and Zinc with preeclampsia. Most of the controls consumed diets rich in calcium in comparison to the cases.

Conclusion: Pregnant women with preeclampsia have lower serum levels of calcium and vitamin D, and are less likely to consume specific foods rich in these micronutrients. There is no association between serum level of selenium and zinc and preeclampsia.

Recommendations: Calcium and vitamin D supplementation for pregnant women thought to be deficient in these micronutrients or who are at risk of preeclampsia is recommended. Pregnant women should receive nutritional advice to encourage deliberate consumption of locally available foods known to be rich in these micronutrients such as milk, natural yoghurt, green vegetables, fish and eggs.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

INTRODUCTION

Preeclampsia (PE) is an unpredictable pregnancy specific condition characterized by new onset hypertension and either proteinuria or end organ dysfunction after 20 weeks gestation in a previously normotensive woman [1]. PE and related hypertensive disorders of pregnancy continue to be a global problem. They constitute the top 5 causes of maternal mortality in the world [2]. Worldwide, 76,000 pregnant women and 500,000 babies die each year from PE and related hypertensive disorders [3]. In developing countries, 15-20% of maternal mortality is attributable to PE [4].

Not only is PE associated with maternal and fetal morbidity and mortality, but also women with PE have an increased risk of developing stroke, hypertension and ischemic heart disease later in life [5, 6, 7], their daughters have the risk of developing the same complications [8] and children who are more likely to develop hypertension as adults [9]. The incidence of preeclampsia alone in the world ranges from 2-8% [10]. In Kenya, the incidence varies from 1.5-9% [11, 12, 13].

Preeclampsia has a complex pathophysiology and the search for finding the causative factors of this disorder continues to interest obstetricians. In spite of several studies in the past decades, the causes remain unknown. Recently the role of oxidative stress (diminished anti-oxidant capacity) and excessive lipid peroxidation in relation to the status of trace metals has been implicated in the pathogenesis of preeclampsia [14, 15, 16]. Malnutrition has been thought to contribute to the

high rate of preeclampsia in developing countries due to the resultant deficits of certain trace elements [17]. While studying Isfahanian pregnant women, [18] established a link between low dietary intake of foods containing calcium, zinc among others and subsequent development of pregnancy induced hypertension.

Low levels of calcium have been observed in women with preeclampsia [19, 20]. For example, Sukonpan and Phupong [19] found that patients with preeclampsia had levels of 9.0±0.4mg/dl of calcium compared to their normotensive counterparts who had higher levels of 9.7±0.7mg/dl. PE is also associated with abnormal concentrations of Zn, Cu and Se such that, these levels are significantly low in women with preeclampsia [21, 22, 23, 20]. Low maternal serum of vitamin D has been documented as a risk factor for occurrence of PE [24].

Part of the preventive strategies of PE includes identification of region specific risk factors. Low levels of certain trace elements and other nutritional deficiencies are established risk factors for PE [25], their supplementation lowering the risk [24]. Some trace elements, such as calcium for example, play a crucial role in production of Nitric oxide, a critical antioxidant, thus preventing PE [26]. The extent of involvement of these mineral deficiencies to PE risk is not yet known in our setup.

Nutritional deficiencies are known to differ from population to population. Women in the developing countries are thought to consume diets that are low in minerals and vitamins [27, 28]. Even within Kenya, macro and micro nutrient deficiencies have been documented to vary from region to region among women [29]. Poor quality diet isn't unusual among Kenyans, case in

point, women attending antenatal clinic in Nakuru, Kenya were found to have poor food regimes [30] while another study reported vitamin D deficiency as high as 79.4% among urban pregnant women [31]. This study looked into the serum levels of calcium, selenium, zinc, and Vitamin D in primigravida with preeclampsia as compared to that with normotensive pregnancies at the Kenyatta National Hospital.

LITERATURE REVIEW

PE continues to be a global burden and is a main contributor to maternal mortality and perinatal morbidity and mortality. Ten thousand women develop PE each year round, 76000 of them die yearly from PE and related disorders while 500000 babies are lost to PE yearly [3]. Fifteen percent of preterm babies are as a result of PE. This burden of preeclampsia is even worse in developing countries. WHO estimates that the number of new cases of preeclampsia to be seven times in low resource countries, 2.8% of live births, than the high resource countries, 0.4% of live births [32]. Prevention of any disease process requires knowledge of risk factors as well as the availability of methods for prediction of those at high risk for that disorder [33].

Risk factors and pathophysiology of preeclampsia

Preeclampsia and eclampsia remain an enigmatic set of conditions whose mechanisms are not clear yet, however, several factors are known to play a part in determining who will develop this disease [3]. Recognized risk factors for PE include nulliparity, family history or own history of PE, pre-existing diabetes or increased body mass index [34], polycystic ovary syndrome [35], high altitude [36], mental stress during pregnancy [37], multiple pregnancy, extremes of maternal age, renal disease, hypertension, chronic autoimmune diseases and thrombophilias [38, 37, 39].

Ethnicity is yet another risk factor for preeclampsia. There appears to be a disparity in incidence in ethnic subgroups, as among primiparous women, the risk of preeclampsia is doubled in black mothers compared with white mothers [40]. Supporting this, there is also an increased risk in

women of Indian and Pakistani origin [41]. Intriguing risk factors related to semen familiarity such as short length of cohabitation before conceiving [42] and change of paternity [43, 44] have also been reported. Indeed, in support of the concept of the familiar sperm, practices that make a female familiar with paternal sperm such as oral sex or swallowing sperm were reported as being protective [45].

Low socioeconomic status and low education status are other strong risk factors for preeclampsia [46, 39]. Another modifiable risk factors are nutritional deficiencies [25]. Nutritional deficiencies are thought to be associated with low socioeconomic status, low education level and being in a developing country [47]. Nutrient status including increased serum triglyceride and fatty acids, and reduced levels of serum calcium, vitamin D, magnesium, and zinc are associated with increased risk of preeclampsia [48]. Abnormal nutrient levels result in inflammation, dyslipidemia and increased oxidative stress, which result in the cascade of pathophysiology of preeclampsia [48].

Pre-eclampsia has a complex pathophysiology, the primary cause being abnormal placentation. Defective invasion of the spiral arteries by cytotrophoblast cells is observed during pre-eclampsia. During normal pregnancy, the villous cytotrophoblast invades into the inner third of the myometrium, and spiral arteries lose their endothelium and most of their muscle fibers. These structural modifications are associated with functional alterations, such that spiral arteries become low-resistance vessels, and thus less sensitive, or even insensitive, to vasoconstrictive substances. In pre-eclampsia, this differentiation process goes amiss such that the spiral arteries maintain high resistance [49]. Increased uterine tone resistance induces higher sensitivity to

vasoconstriction and thus chronic placental ischemia. This chronic placental ischemia causes fetal complications such as intrauterine fetal growth restriction and intrauterine death. In parallel, oxidative stress induces release into the maternal circulation of substances such as free radicals, oxidized lipids, cytokines, and serum soluble vascular endothelial growth factor 1. These abnormalities are responsible for endothelial dysfunction [50] with vascular hyperpermeability, thrombophilia, and hypertension, so as to compensate for the decreased flow in the uterine arteries due to peripheral vasoconstriction.

Once endothelial dysfunction has occurred, it results in the clinical signs observed in PE, such as, impairment of the hepatic endothelium contributing to onset of the HELLP (Hemolysis, Elevated Liver enzymes and Low Platelet count) syndrome, impairment of the cerebral endothelium inducing refractory neurological disorders, or even eclampsia. Depletion of vascular endothelial growth factor in the podocytes makes the endotheliosis more able to block the slit diaphragms in the basement membrane, adding to decreased glomerular filtration resulting in proteinuria. Additionally, endothelial dysfunction promotes microangiopathic hemolytic anemia, and vascular hyperpermeability associated with low serum albumin causes edema, particularly in the lower limbs or lungs [51].

Several theories are thought to play a role in the pathophysiology of preeclampsia: the oxidative stress theory [52], the immunological [53] and the genetic theory [4]. The oxidative stress theory is about an imbalance between maternal prooxidants and antioxidants; this imbalance precedes the clinical recognition of the syndrome [52]. Diet is thought to play a role in this imbalance [52]. Several susceptibility genes may exist for pre-eclampsia. These genes are said to interact in

the hemostatic and cardiovascular systems, as well as in the inflammatory response. Some have been identified, and in candidate gene studies they have provided evidence of linkage to several genes, including angiotensinogen on 1-q42–43 and eNOS on 7q36; other main important loci are 2p12, 2p25, 9p13, and 10q22.1. Individual with such genes have a higher likelihood of developing PE [54].

Preeclampsia has been perceived as having an impaired maternal immune system exhibiting excessive production of immune cells, which cause secretion of tumor necrosis factor alpha that induces apoptosis of the extravillous cytotrophoblast [55]. The human leukocyte antigen (HLA) system also appears to play a role in the defective invasion of the spiral arteries, in that women with pre-eclampsia show reduced levels of HLA-G and HLA-E [53]. During normal pregnancies, the interaction between these cells and the trophoblast is due to secretion of vascular endothelial growth factor and placental growth factor by natural killer cells. High levels of soluble fms-like tyrosine kinase 1 (sFlt-1), an antagonist of vascular endothelial growth factor and placental growth factor, have been found in women with pre-eclampsia [53, 55].

Nitric oxide (NO) and endothelin 1 imbalance is the final common pathway in the pathogenic cascade of preeclampsia [56, 57]. NO contributes substantially to the control of vascular tone to make the vessels relax. In the absence of normal nitric oxide levels, increased uterine arterial resistance occurs [49], as a result of failure of compensatory mechanisms that produce NO [57]. To date the only cure of preeclampsia is removal of the placenta since it occurs only in the presence of a placenta and its resolution begins with removal of the placenta [58].

Calcium

The maternal demand for calcium (Ca) during pregnancy is elevated by as much as 300 mg/d to provide the calcium necessary for fetal bone mineralization [59]. The normal expansion of maternal blood volume and the pregnancy-induced increase in urinary calcium excretion that occur in well-nourished women add further to the physiologic calcium requirement [60]. This additional calcium is normally provided by an increase in maternal intestinal calcium absorption. Low serum calcium may cause high blood pressure by stimulating parathyroid hormone and renin release and also by inducing vasoconstriction by increasing its level in vascular smooth muscle [61]. Calcium might also have an indirect effect on smooth muscle function by increasing magnesium levels [62]. Magnesium on the other hand plays an important role in peripheral vasodilatation [63].

Various case control studies have documented low levels of calcium in women with preeclampsia in different regions: Ghana [64], India [65, 66], Kerman-Iran [20], Tehran-Iran [67], Saudi Arabia [68], Sudan [69] and Korea [70], as illustrated in the table below. On the contrary, Golmohammad et al. [71] established that serum levels of calcium, including zinc, magnesium and copper did not differ significantly in preeclamptic compared to normotensive Iranian women. Richards et al. [72] also reported the same in South Africa. According to this study, the supplementation of calcium doesn't reduce preeclampsia by correcting a nutritional deficiency since the women in their population portrayed similar serum and hair levels of calcium [72].

Table 1: Serum levels of Calcium in different regions comparing preeclamptic and normotensive groups

Authors	Region	Serum Ca in PE group (mg/dl)	Serum Ca in Normotensive group (mg/dl)	P value
Al Jameil et al. [68]	Saudi Arabia	7.78 ± 0.44	9.00 ± 0.63	> 0.05
Kanagal et al. [66]	India	7.84 ± 0.87	8.97± 0.69	<0.001
Abdella and Adrabo [73]	Sudan	7.56 ± 0.82	8.69 ± 0.34	0.000
Farzin and Sajadi [67]	Iran	8.65 ± 2.14	9.77 ± 3.02	<0.01
Mohieldein et al. [69]	Sudan	8.34 ± 1.04	9.04 ± 1.13	0.001
Sukonpan and Phupong [18]	Thailand	9.0 ± 0.40	9.7 ± 0.70	<0.001

In 2011 the World Health Organization recommended calcium supplementation with 1.5–2.0 g elemental calcium daily for pregnant women in areas with low dietary calcium [74]. Differences in dietary calcium intake between low-income and high-income countries approximate 500 mg. Typical daily intake in low-income countries ranges between 300 and 600 mg/day, compared with 855 mg (UK) and 969 mg (France) (FAO-UN 1991). The status of calcium among pregnant women in Kenya is unknown. All the same, there are no studies comparing women with preeclampsia or those without. Antenatal calcium supplementation is not a uniform practice in our country. The findings of this study may contribute in directing antenatal practices regarding calcium.

Zinc

Zinc (Zn) is an essential mineral known to be important for many biological functions including protein synthesis, cellular division and nucleic acid metabolism [75]. Zinc is also an anti-oxidant and even more so at supraphysiological levels. It does so by inhibiting the production of reactive oxygen species [76]. Its deficiency is uncommon but can occur in populations with low consumption of zinc-rich animal-source foods and high intakes of foods rich in phytates, which inhibit zinc absorption. It is estimated that over 80% of pregnant women worldwide have inadequate zinc intake [77].

Low levels of zinc are related to the risk of developing gestational hypertension and preeclampsia, women with these conditions exhibiting lower levels of this element [78, 70, 67, 79, 20]. This is indicated in the table below. On the other hand, ensuring adequate dietary intake of zinc is thought to help prevent development of hypertensive disorders of pregnancy [79]. Nonetheless, zinc supplementation has little beneficial effects in preeclampsia prevention. For this, reason routine supplementation is not recommended and improvement of overall nutritional status of women in low-income setting may be more prudent as is recommended for children [80]. In extensive search of literature, there are no studies on zinc status among pregnant women in Kenya.

Table 2: Serum levels of zinc in different regions comparing preeclamptic and normotensive groups

Authors	Region	Serum Zn in PE group (mg/l)	Serum Zn in Normotensive group (mg/l)	P value
Al Jameil et al. [68]	Saudi Arabia	0.67± 0.59	1.30 ± 0.83	<0.001
Farzin and Sajadi [67]	Iran	0.76 ± 0.17	1.00 ± 0.20	0.001
Akhtar et al. [21]	Bangladesh	0.90 ± 0.16	1.15 ± 0.07	<0.001
Akinloye et al. [81]	Nigeria	0.56 ± 0.09	0.61 ± 0.05	< 0.05

Selenium

Selenium (Se) is an essential micronutrient that acts as an antioxidant to protect the cells from generating free radicals. It constitutes an integral part of approximately 20 enzymes (selenoproteins). One such enzyme is Glutathione peroxidase, which participates in metabolism of hydrogen peroxide and plays a protective role against lipids oxidation [82]. Selenium rich foods include: seafood (white, oily and shellfish, fish fingers, and fish roe); meat (beef, beef burgers, pork and lamb, bacon, ham, sausages, and corned beef); poultry; Brazil nuts; offal (liver and liver products) and dairy products [83]. Selenium obtained through the diet has regional variations in intake, which has been correlated geographically with soil selenium concentration, geochemistry, and rainfall [84].

The role of selenium and endogenous antioxidant proteins in the development and progression of preeclampsia is gaining favor in line with the oxidative stress hypothesis [85]. Furthermore, other selenoproteins, which are depleted in preeclampsia, are said to worsen the development and progression of the syndrome. In their review of literature, Vanderlie and Perkins [85] found

a negative correlation between Se status and the incidence of PE in an epidemiological study of forty-five countries. Significantly, lower levels of selenoenzymes such as glutathione peroxidase and thioredoxin reductase have been found in serum, plasma and placenta samples from pre-eclamptic women than in those from matched healthy controls [86]. Other studies have demonstrated low levels of serum selenium in preeclamptic females compared to their normotensive counterparts as shown in the table below.

Table 3: Serum levels of selenium in different regions comparing preeclamptic and normotensive groups

Authors	Region	Serum Se in PE group (µmol/l)	Serum Se in Normotensive group (µmol/l)	P value
Ghaemi et al. [87]	Iran	0.89 ±0.03	1.03 ± 0.01	<0.05
Farzin and Sajadi [67]	Iran	1.11± 0.03	1.33 ± 0.35	<0.01
Akinloye et al. [81]	Nigeria	0.6 ± 0.1	1.3 ± 0.41	<0.001

In response to the selenium deficiencies in different regions, countries such as Finland and New Zealand have instituted compulsory selenium supplementation [88]. European countries differ in incidences of preeclampsia; those with higher selenium intake exhibiting lower incidences. The routine supplementation of selenium in New Zealand and Finland provides an important example of direct intervention in the food chain. Both these countries have been successful in increasing the selenium status of residents above the 95µg/L level and the current analysis demonstrated an associated significant reduction in the reported incidence of preeclampsia [85].

There are limited studies available that report selenium supplementation during pregnancy. However, all published studies available to date have reported reductions in the incidence of

hypertensive complications of pregnancy in those patients with increased selenium intake. Selenium content in soil is known to differ from region to region in our country affecting levels of trace elements in wildlife [89], however its status in pregnant women and involvement with preeclampsia remains unknown. The outcomes of this study may give a directive on way forward regarding this mineral and its contribution to PE.

Vitamin D

Vitamin D is a seco-steroid pro-hormone which, for biological activation, undergoes two successive hydroxylations, firstly to 25-hydroxyvitamin D (25(OH) D), a nutritional biomarker for vitamin D status, and secondly to the active hormonal metabolite 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), i.e. calcitriol. Calcitriol exerts the hormonal action via binding to nuclear vitamin D receptors, which are present throughout the body, including pregnancy-specific tissues such as the placenta and uterine placental bed (decidua). The placenta and decidua as well as other important target cells such as immune and endothelial cells have the molecular machinery for local production of calcitriol [90].

Vitamin D deficiency is a widespread public health problem. Generally, it is more prevalent in black than white populations. In the United States, it was found that approximately 29% of Black pregnant women and 5% of white pregnant women residing in the northeastern United States had vitamin D deficiency, *i.e.* serum 25-hydroxyvitamin D [25(OH) D] of less than 37.5 nmol/liter, whereas 54% of Black women and 47% of white women had serum 25(OH) D levels indicative of vitamin D insufficiency, *i.e.* 25(OH) D of 37.5–80 nmol/liter [91]. The reason for this is attributed to the deeply pigmented skin. In Kenya, the prevalence of vitamin D deficiency in an

urban obstetric population is much higher at 79.4% [31]. This high prevalence prompted the author to recommended routine supplementation of vitamin D among pregnant women in Kenya. This is not routinely practiced in Kenya.

Low maternal vitamin D levels are associated with an increased risk of preeclampsia and is said to be an independent risk factor for this condition [91, 92]. Patients with preeclampsia show low levels of vitamin D compared to normotensive women matched for gestation and age [93, 94, 95]. These values notably vary from region to region. Some authors believe that the much higher prevalence of vitamin D deficiency in African countries is related to the higher prevalence and severity of preeclampsia observed in these countries [91]. Given the high prevalence of vitamin D deficiency in Kenya, this study explored its association with preeclampsia.

JUSTIFICATION

Identification of an effective strategy to prevent PE remains a priority and a challenge in research in obstetrics. Research is now focusing on prevention rather than treatment. There is evidence that indicates a role for micronutrients supplementation in preventing some pregnancy disorders such as preeclampsia [62]. In the past decade the role of general nutritional deficiency and imbalance of specific nutrients have been emphasized in the etiology of the disease [48], research on the same remains limited [96], especially in Kenya. There is inadequate evidence in support of nutrient supplementation to reduce the risk of PE, with the exception of calcium supplementation [97].

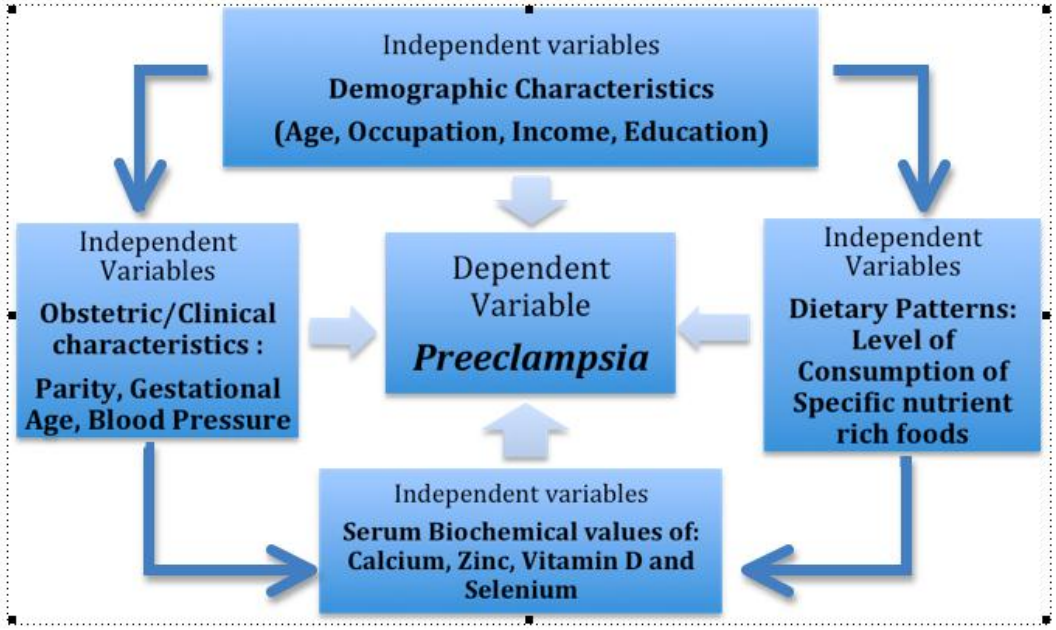
More studies on the role nutrient deficiencies are required. Women of reproductive age group remain susceptible to both macro and micro nutrient deficiencies and the risk is increased in pregnant women due to increased requirement of nutrients to fulfill the need of the growing fetus [75]. Nutritional deficiencies are more common in resource poor setting, especially Africa. A recent cross-sectional study on Vitamin D levels in urban pregnant Kenyan women revealed a deficiency of more than 70% [31]. Given, the need to help prevent preeclampsia being a global priority and evidence of the link of these deficiencies with preeclampsia, we found it essential to explore the contribution of these micronutrients to PE in our setup.

SIGNIFICANCE OF THE STUDY

Little information is available in our region on the contribution of vitamin D and the select trace elements in development of PE. The findings of this study may help acknowledge trace element deficiency in our setup and depending, inform the need for food fortification. The outcomes of this study may also influence preconception and antenatal care as well as advice on specific nutrient supplementation.

CONCEPTUAL FRAMEWORK

Figure 1: Conceptual Framework: Relationship between dependent and independent Variables



This framework displays the interaction between the dependent variable (preeclampsia) and the independent variables (demographic characteristics, Serum levels of calcium, Zinc, Vitamin D and Selenium, Dietary patterns and Obstetric Characteristics). Low serum levels of the select micronutrients have been shown to be a predisposing factor to preeclampsia. On the other hand, reduced consumption of specific nutrient rich foods affects the serum levels of the micronutrients. Demographic characteristics such as extremes of age, low level of income and education are independent risk factors to the occurrence of preeclampsia, and also influence dietary patterns. These demographic features such as age, level of education and income also affect parity of women as well as their blood pressure during pregnancy. Gestational age may also be influenced by some demographic characteristics, for example, level of education of a woman which may affect their antenatal seeking behavior and therefore pregnancy dating. Blood

pressure during pregnancy, parity and gestational age influence the occurrence of preeclampsia and is also related to serum levels of micronutrients. This study fixed the parity to primigravida as a way of reducing confounders with a small sample size.

RESEARCH QUESTION

How do the serum levels of the select micronutrients (Vitamin D, calcium, zinc and selenium) differ in women with preeclampsia compared to their normotensive counterparts?

NULL HYPOTHESIS

There is no difference in serum levels of Vitamin D and select elements (calcium, zinc and selenium) between primigravid women with preeclampsia and the normotensive ones.

OBJECTIVES

Broad Objective:

To investigate the serum levels of the select micronutrients (Vitamin D, Selenium, Calcium, Zinc) in preeclamptic and normotensive primigravida

Specific objectives:

1. To compare the serum levels of select micronutrients (Ca, Zn, Se and Vit. D) in preeclamptic and normotensive primigravida.
2. To correlate the serum levels of these micronutrients with age groups, level of income, level of education and severity of disease in the different studied groups
3. To determine the level of consumption of specific nutrient rich foods among the cases and controls

CHAPTER TWO

MATERIALS AND METHODS

Study Design:

This was a case-control study in which 54 primigravida with preeclampsia (cases) were compared with 54 primigravida who were normotensive (controls) and were matched for gestation and age.

Study Setting:

The study was conducted at the Reproductive Health Department of the Kenyatta National Hospital, a regional teaching and referral hospital with an 1800 bed capacity. This hospital has a busy reproductive health department that conducts 20 – 50 deliveries per day. The prevalence of preeclampsia in this institution is 5%. The labour ward is the initial place where most patients with preeclampsia are admitted, some of them eventually would be transferred to the antenatal wards. If seen at the antenatal clinics, they would be admitted to the antenatal wards if conservative management is instituted from the clinic. The cases were therefore recruited at the labour ward and the antenatal wards. The normotensive controls were recruited from the antenatal wards and antenatal clinics.

Study population:

A hundred and eight primigravid women comprised the study population. These included primigravida with preeclampsia ≥ 20 weeks gestation who were compared with their normotensive counterparts matched at the same gestation and age. These were 54 cases and 54

controls matched for age (±2years) and gestational age (± 2 weeks). They were recruited between 1st of March 2016 and 30th of June 2016

Sample size

Sample size (n) is estimated as shown below as described by Kasiulevicius et al. [98].

$$n = (\frac{r+1}{r}) \frac{\sigma^2 (Z_\beta + Z_{\alpha/2})^2}{(\mu_{Cases} - \mu_{Controls})^2}$$

This formula gives the minimum number of case subjects required to detect a true mean difference of serum levels of Zinc with power (1- β) and two-sided type I error probability α (alpha).

Where

n is number of case subjects.

$\mathbf{Z_{\alpha/2}}$ is the desired level of statistical significance (typically 1.96 for 95% confidence level)

$\mathbf{Z_{\beta}}$ is the desired power (typically .84 for 80% power)

r is the number of control subjects per experimental subject = 1

σ is the standard deviation of the serum levels of zinc =1.1

$\mu_{Cases} - \mu_{Controls}$ is the desired difference of serum levels of zinc to be detected in the two groups also known as effect size=0.7

Therefore,

$$n = \left(\frac{1+1}{1}\right)\frac{1.1^2\left(0.84+1.96\right)^2}{\left(0.7\right)^2} = 38.6$$

Therefore, n=39 (39 cases, 39 controls)

Since doing subgroup analysis for the cases was anticipated, the sample size was increased by 40%, and to also cater for loss of information due to either participant attrition or lack of response. The adjusted sample size was 39+15=54 rounded of to (54 cases, 54 controls). The above formula was applied to all the trace elements as well as vitamin D, but Zinc gave the highest sample size and was therefore used to obtain the representative sample.

Sampling method

This study used the purposive sampling method. The first 54 patients with the preeclampsia spectrum of disease (mild disease, severe disease) in their first pregnancy who fit the inclusion criteria were included in the study matched for gestational age with normotensive controls.

Inclusion criteria

Pregnant women after 20 weeks of gestation were included in this study. The control group comprised pregnant women with normal BP, absence of proteinuria, normal renal function (GFR of less than 170ml/min or creatinine of less than 1.1mg/dL) and without any other systemic or endocrine disorder and gestational age of above 20 weeks. Selection of the preeclamptic group (cases) was according to the definition by the American College of Obstetrics and Gynecologists: ACOG [1], see Table 4. Patients with severe disease, HELLP and

any other organ failure as a result of preeclampsia severity were also included in the study.

Severe preeclampsia is defined by the presence of one or more of the following, using the ACOG [1], criteria; systolic BP ≥ 160 mm Hg or diastolic ≥ 110 mm Hg on 2 occasions 4 hours or more apart while the patient is on bed rest, Thrombocytopenia (platelet count < 100,000/μL), impaired liver function as indicated by abnormally elevated blood levels of liver enzymes (to twice normal concentration), severe persistent right upper quadrant or epigastric pain unresponsive to medication and not accounted for by alternative diagnoses, or both, progressive renal insufficiency (serum creatinine > 1.1 mg/dL or a doubling of the serum creatinine in the absence of other renal disease new-onset cerebral or visual disturbances and pulmonary edema. **Eclampsia** is defined as the presence of new-onset grand mal seizures in women with preeclampsia while **HELLP Syndrome** is characterized by hemolysis, elevated liver enzymes, and low platelets, hence the acronym.

Table 4: Diagnostic Criteria For Preeclampsia (ACOG 2013) [1].

Blood pressure	• ≥ 140 mm Hg systolic or ≥ 90 mm Hg diastolic pressure on 2 occasions at least 4 hours apart after 20 weeks GA in women with a previously normal BP • ≥160mmHgsystolic or≥110mmHg diastolic, confirmed within a short interval (minutes) to facilitate timely antihypertensive therapy
And	
Proteinuria	≥ 300mg per 24-hr urine collection (or this amount extrapolated from a timed collection) Or Protein/creatinine ratio ≥ 0.3 mg/dL Dipstick reading of ≥ 1+ (used only if other quantitative methods not available)
Or in the absence of proteinuria, new-onset hypertension with the new onset of one or more of the following:	
Thrombocytopenia	Platelet count<100,000/μL
Renal insufficiency	Serum creatinine>1.1mg/dL or a doubling of the serum creatinine in the absence of other renal disease
Impaired liver functions	Elevated blood levels of liver transaminases to twice normal concentrations
Pulmonary oedema	
Cerebral or visual symptoms	

Exclusion criteria

Patients with any one of the following parameters were excluded from our study: fetal abnormalities, chronic diseases, infections, immunological disorders, alcohol or drug abuse, smoking, history of infertility, obstetric complications such as placental abruption or previa, consumption of anti-cancer, immunosuppressive and anticoagulant drugs.

Data Sources

Data was obtained from questionnaires for demographic data (Age, Religion, Residence in the past year Marital Status, Occupation), Obstetric history (Parity, birth interval, Dating, Paternity) and for dietary assessment as indicated below. Blood was drawn for biochemical assessment as explained below.

Dietary intake assessment

Dietary intake, for some foods rich in the micronutrients of concern, was assessed by means of a semi-quantitative food frequency questionnaire (FFQ) and 24-hour dietary recall, which was filled by a trained research assistant. Participants were asked to report their frequency of consumption of each food item during the previous year on a daily, weekly or monthly basis. Routine daily or weekly intake was classified as frequent, while monthly, occasional or once in a while was classified as infrequent.

Collection of blood and biochemical analysis

Venous blood samples (5ml) were obtained from all cases and controls using sterile disposable syringes. The blood samples taken were put in specimen bottles with a serum separator gel (Fig. 1) and taken to a diagnostic laboratory in Nairobi for analysis. After centrifugation to obtain the serum, calcium was measured by atomic absorption spectrophotometry. Albumin levels were

also obtained for all patients in order to calculate the corrected calcium according to serum albumin levels. Corrected calcium was calculated in millimoles per litre (mmol/l) using the formula: corrected calcium = measured total calcium (mmol/l) + 0.02[40 – serum albumin (g/l).

Figure 2: This figure shows the serum separating tubes, which contain a special gel (*) that separates blood cells from serum as well as particles that cause blood to clot quickly.

Zinc and selenium were determined through Inductively Coupled Plasma Mass Spectrometry (ICP MS). ICP MS is a type of mass spectrometry, which is capable of detecting metals and several non-metals at concentrations as low as one part in 10^{15} (part per quadrillion) on non-interfered low-background isotopes. This is then achieved by, ionizing the serum sample with inductively coupled plasma and then using a mass spectrometer to separate and quantify the ions.

The serum level of vitamin D was obatined via electrochemiluminescence immunoassay (ECLIA). ECLIA uses the principle of incubating Vitamin D in an antibody coupled with a luminescence substance, capable of emitting light, such as Ruthenium. This complex is then bound to streptavidin-coated micro-particles and the unbound particles are washed away. The

remaining mixture is measured using a photomultiplier. These tests were carried out at a private laboratory, Lancet. This laboratory was selected to ensure internal quality control and its willingness to take the responsibility of transporting the non-routine tests (zinc and selenium) for analysis out of the country with a maximum duration of 2 weeks to obtain results. The serum values of Ca, Zn and Se were interpreted in accordance to the expected normal control values in pregnancy for pregnant women in the second and third trimesters (Table 5). The serum vitamin D values were interpreted as follows (RCOG 2014) [99]:

- Deficient levels ≤ 20ng/ml
- Insufficient levels- 21-29ng/ml
- Preferred value ≥ 30ng.ml

Table 5: Illustration of the normal ranges of the serum levels of Ca, Zn and Se in pregnancy

Micronutrient	**Normal range**	
	Second trimester (13-26 weeks)	**Third trimester (>27 weeks)**
Calcium	2.05mmol/l to 2.25mmol/l	2.05mmol/l to 2.43mmol/l
Selenium	75mcg/l to 145mcg/l	71mcg/l to 133mcg/l
Zinc	7.8µmol/L to 12.2µmol	7.6µmol to 11.8µmol

Statistical analysis

Data on demographic and obstetric parameters, nutritional patterns and biochemical parameters were entered into SPSS version 21. Descriptive statistics such as means of the biochemical parameters was obtained for the two groups and compared using the Students T test, a P value of <0.05 was considered statistically significant. Categorical variables were compared using Chi

square test, a P value of <0.05 being statistically significant, when the samples within the categories are less than 5, the Fisher's exact test was used.

ETHICAL CONSIDERATIONS

Number of Participants

A hundred and eight women, 54 patients with preeclampsia and 54 normotensive ones participated in this study. Five milliliters (5ml) of blood was obtained from these patients as described in the methodology section for biochemical analysis. Individual interviews were done to know the demographic, obstetric and dietary habits of these women.

Confidentiality

The names of patients were not indicated on the questionnaire. We used hospital numbers as identifiers as well as their initials. Password protected documents were used to keep the information collected from the study private. Patients who refused to participate in the study were not victimized.

Informed consent

We explained the purpose of the study to the patients, in a language best understood to them, including the blood collection procedure. We also explained that, should they chose to participate in the study, they will sign consent; have information regarding their demographic characteristics, obstetric and clinical characteristics obtained from them, and the blood samples taken.

Provision of debriefing and counseling and benefits to the participants

Through phone calls, we gave feedback to the patients who participated in this study. We encouraged those with low serum levels of the micronutrients in question to increase intake of specific nutrient rich foods available to them. We also encouraged those with low vitamin D to spend at least 30 minutes in the sun daily.

Training of research Assistants

One research assistant with a background of medical training, a clinical officer, was trained on how to use the questionnaire to obtain the information required. The same assistant was involved in collection of blood sample.

STRENGTH, LIMITATION AND DELIMITATION OF THE STUDY

The findings of this study provide an understanding of the status of Vitamin D, Selenium, Calcium and Zinc among pregnant normotensive and preeclamptic women in our setup and it is the first of its kind. The findings of this study can be generalized locally since the selection process allowed for representation of the study population. The data obtained from this study was easy to analyze, was consistent and reliable and therefore can be reciprocated with ease.

One of the limitations of this study was the practice of give loading dose of MgSO4 at immediate diagnosis of preeclampsia, which is an acceptable exercise at KNH and in other institutions. It was noted that analysis of serum levels of magnesium, which was initially part of the micronutrients to be assessed, revealed a highly statistically significant difference between cases

and controls (Table 6). For this reason, these results were not used in drawing a concrete conclusion regarding serum levels of Magnesium. It was also put into considerations that hypermagnesaemia may affect serum levels of calcium by lowering them. To mitigate this, blood samples were collected within 20 minutes of administration of magnesium sulfate, given that the peak reduction of calcium following hypermagnesaemia occurs between 120-180 minutes [100]

Table 6: Illustration of serum Magnesium levels for the cases and controls

Micronutrient n=108	**Average for the cases**	**Average for the controls**	**P-Value**
Magnesium in mmols/l	1.33±0.5	0.96±0.7	0.005

Normal range of serum Magnesium is: 0.45mmol/l to 0.90mmol/l

HAPTER THREE

RESULTS

Data was collected from1st March 2016 to 30th June 2016 at the labour and antenatal wards as well as antenatal clinics of the Kenyatta National Hospital. The study population comprised 108 primigravida, 54 of whom had preeclampsia while 54 were normotensive.

Demographic characteristics

The mean age was 24.7±4.2 and 25.04±4.9 years for the cases and controls respectively. Most (72%) of the women in the study population were between ages 20-30 years. Almost all (99%) were Christian. Most of the study participants had attained at least secondary education (80%, n=108), with an almost equal distribution between the cases and the controls (see Table 7). Sixty one percent of the patients had an average income of less that 10,000. Most of the patients resided in or within the outskirts of Nairobi. Regarding marital status, 77% of the participants were married. The distribution of these demographic characteristics was similar between cases and controls.

Table 7: Demographic characteristics of the study population

Characteristic		**Total**	**Cases (n=54)**	**Control (n=54)**	**P-Value**
Age distribution: (n=108)	<=20years	17(15.7)	9(16)	8(15)	
	21-30 years	78(72.2)	37(69)	41(76)	
	31-40 years	13(12.1)	8(15)	5(9)	0.62
Religion: (n=103)	Christian	102(99)	50(100)	52(98)	
	Muslim	1(1)	0(0)	1 (2)	-
Education level: (n=108)	Primary	10(9)	4(8)	6 (11)	
	Secondary	86(80)	45(83)	41(76)	
	Tertiary	12(11)	5(9)	7(13)	0.631
Average monthly income (n=106)	<10,000	65 (61)	36 69)	29 (54)	
	>=10,000	41(39)	16(31)	25 (46)	0.101
Marital status: (n=105)	Not married	28(27)	13(25)	15(29)	
	Married	77(73)	40(75)	37(71)	>0.999

Obstetric characteristics

All the patients recruited for this study were in their first pregnancy (primigravida), and had singleton gestation. The average gestational age was 35.2±4.4 weeks for the cases and 35.4±4.4 weeks for the controls. Seven of the 108 were in the first trimester (6.5%), while 101 (93.5%) were in the 2nd trimester (see Table 8). The 7 in the 2nd trimester, 3 were cases and 4 controls. The distribution between cases and controls in the 3rd trimester was 51 (94%) and 50 (93%) respectively. For the cases, 21 (38.9%) had preeclampsia without features of severity (formerly mild preeclampsia) and 33 (61.1%) had severe preeclampsia. The average systolic blood pressure among the cases was 155.7±17.4, while their average diastolic blood pressure was 105 ±10.2. The controls had an average systolic BP of 123.8 ±11.1 while their average diastolic BP was 74.2 ± 7.1.

Table 8: Obstetric and clinical characteristics of the study population

Characteristic		**Cases (Preeclamptics)**	**Controls (Normotensive)**
Average gestational age in weeks		35.2±4.4	35.4±4.4
Average systolic pressure		155.7±17.4	105 ±10.2
Average diastolic pressure		123.8 ±11.1	74.2 ± 7.1
Distribution across trimesters n=108			
Trimester	**Total (%)**	**Cases (%)**	**Controls (%)**
Second trimester	7(6.5)	3 (6)	4(7)
Third trimester	101(93.5)	51(94)	50(93)
Severity of disease among the preeclamptics n=54 (%)			
Mild preeclampsia	21 (39)		
Severe preeclampsia	33 (61)		

Assessemnent of micronutrient deficiency and the mean serum levels of the micronutrients among the cases and controls

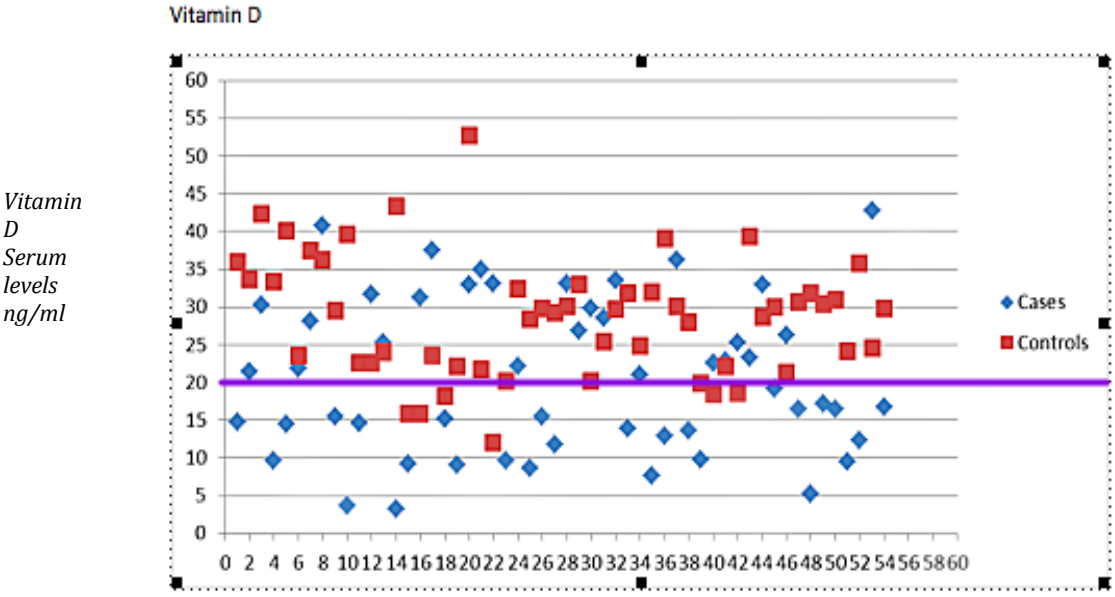
Vitamin D deficiency was more likely to be present among women with preeclampsia compared to the normotensive controls: 50% versus 13% respectively (p=<0.001), Table 8. It was also noted that for the cases, 13(24%) had sufficient serum levels of vitamin D (≥30ng/ml), while the rest 14 (26%), had levels that are considered insufficient (20-30ng/nl) (Fig. 3). On the other hand, 30 (56%) of the controls had sufficient levels of vitamin D, while 17(31%) had insufficient amounts. Only 3 patients had deficient serum levels of calcium, all of whom were among the cases. This was however, not statistically significant. Prevalence of deficiency of zinc and selenium did not show statistically significant differences between the cases compared to controls, (Table 9).

Table 9: Proportion of women with Vit. D, Se, Ca and Zn deficiency among preeclamptic and normotensive primigravida

Micronutrient		**Total n=108 (%)**	**Study groups**		**P-value**
			Cases (%)	**Controls (%)**	
Vitamin D	<20ng/ml	34(31)	27(50)	7(13)	<0.001
	≥20ng/ml	74(69)	27(50)	47(87)	
Selenium	<71mcg/l	13(12)	8(15)	5 (9)	0.375
	≥71mcg/l	95(88)	46(85)	49 (91)	
Calcium	<2.05mmol/l	3(3)	3 (6)	0	0.243
	≥2.05mmol/l	105(97)	51(94)	54(100)	
Zinc	<7.6μmol	24(22)	13(24)	11(20)	0.643
	≥7.6μmol	84(78)	41(76)	23(80)	

The vitamin D level was significantly lower among the cases than the controls ($p<0.001$). Note that most of the cases (blue diamonds) have levels below 20ng/ml (purple line), while the controls (red squares), have levels above 20ng/ml

Figure 3: Distribution of serum vitamin D among preeclamptics and normotensive controls.



The study found that, the mean serum levels of vitamin D and calcium were significantly lower among the cases than the controls. Mean serum Vitamin D was 20.8 ± 10.2 for the preeclamptics while it was 28.2±7.9 for the normotensive ones ($p<0.001$). Mean zinc serum levels were lower among the cases but this was not found to be statistically significant (Table 10). On the other hand the average serum selenium was higher among the cases than the controls. This observation was found not to be statistically significant.

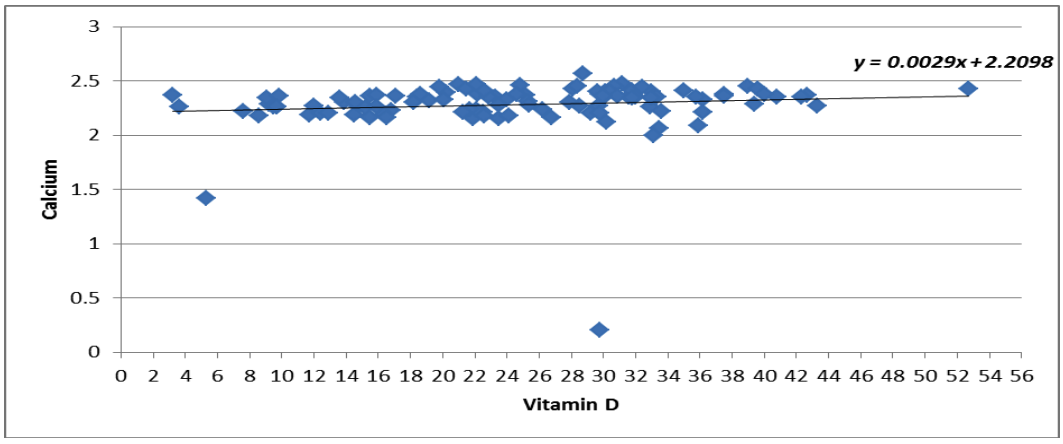
Table 10: Distribution of the mean serum levels of the micronutrients among the preeclamptic and normotensive primigravida

Micronutrient	Study group ± SD		P-Value
	Cases (n=54)	Controls (n=54)	
Vitamin D	20.8±10.2	28.6±7.9	<0.001
Selenium	103.5±33.3	99.7±24.7	0.498
Zinc	9.9±3.7	10.7±3.5	0.28
Calcium	2.2±0.3	2.3±0.09	0.024
Magnesium	1.33±0.5	0.96±0.7	0.005

The mean serum levels of vitamin D and calcium were significantly lower among the cases in comparison to the controls.

The relationship between serum calcium and serum vitamin D for the women with preeclampsia revealed a weak positive correlation between them, such that those with low calcium are more likely to also have low vitamin D serum levels. This correlation was not statistically significant (Pearson Correlation= 0.121, P-value=0.214) see Figure 4.

Figure 4: Correlation between serum levels of vitamin D and calcium among the study population



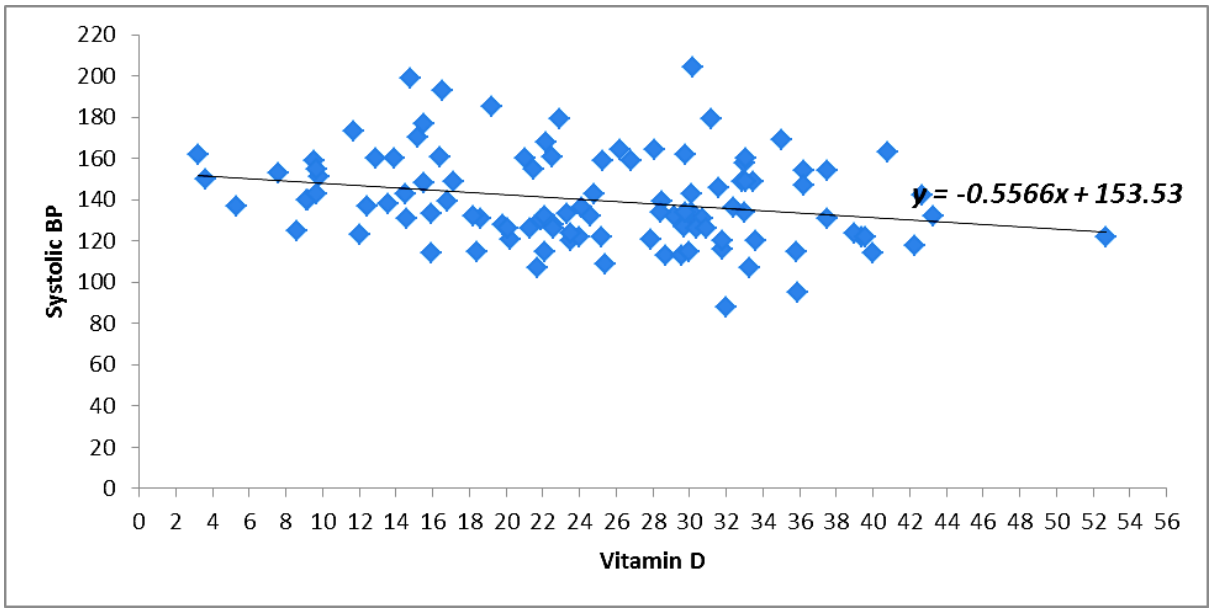
Pearson Correlation= 0.121

P-value=0.214

Association of serum means of the micronutrients with blood pressure, disease severity, age, level of income and education status

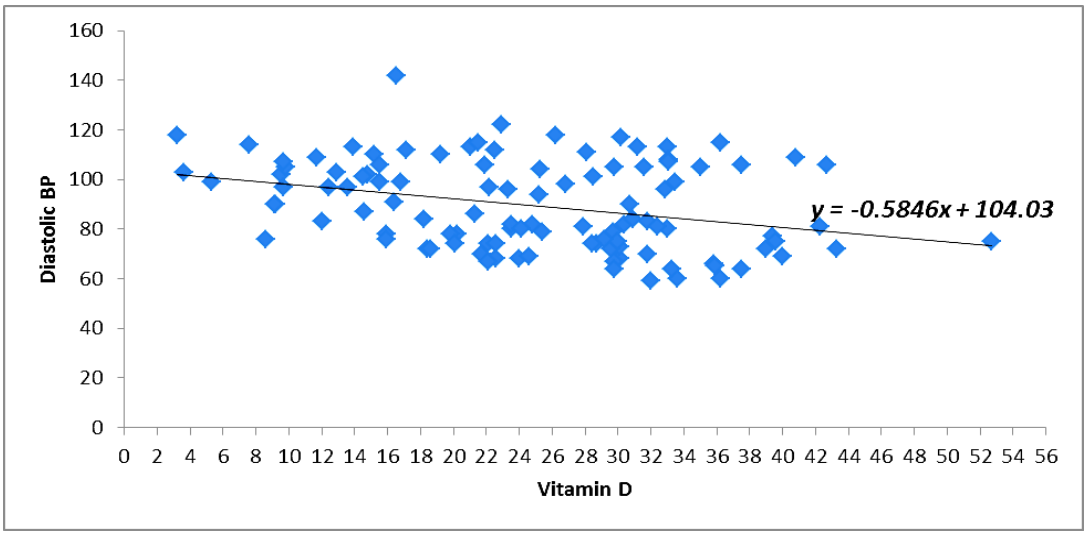
The correlation of serum levels of vitamin D and calcium with systolic blood pressure (SBP) and diastolic blood pressure (DBP) were obtained. It was observed that there was a small negative correlation between both SBP and DBP and serum vitamin D levels such that, the lower the vitamin D levels, the higher the SBP (Pearson correlation=-0.255, P-value=0.008) and the same for DBP (Pearson correlation=-0.326, P-value=0.001). These observations were statistically significant. (Figures 5 and 6)

Figure 5: Correlation between serum vitamin D and systolic blood pressure among the study population



Pearson correlation=-0.255, P-value=0.008

Figure 6: Correlation between serum vitamin D and Diastolic blood pressure for the study population



Pearson correlation=-0.326, P-value=0.001

There was a weak negative correlation between serum calcium and SBP as well DBP, such that the higher serum calcium the lower the blood pressure and the reverse, but it was not statistically significant. The correlation between serum calcium and SBP was -0.152, p = 0.117, while for serum calcium and DBP was -0.159, p= 0.101. There was no correlation between selenium and SBP (0.019, p = 0.842), selenium and DBP (= 0.092, p = 0.346), zinc and SBP (-0.056, p = 0.560) and zinc and DBP (-0.054, p= 0580).

Regarding disease severity, there was no difference in mean serum levels of the micronutrients (vitamin D, selenium, zinc and calcium) among the patients with the mild preeclampsia compared to those with severe preeclampsia group (Table 11).

Table 11: Mean serum levels of Vit. D, Se, Zn and Ca among the Preeclamptics, with mild preeclampsia (without severe features) and severe preeclampsia

Micronutrient	**Study group**		**P value**
	Mild PE	**Severe PE**	
Vitamin D (ng/ml)	19.9±11.0	21.3±9.7	0.646
Selenium (mcg/l)	96.6±29.7	107.9±35.1	0.227
Zinc (μmol/l)	10.5±3.0	9.6±4.1	0.415
Calcium (mmol/l)	2.2±0.2	2.2±0.4	0.751

The serum levels of zinc and selenium were observed to be significantly lower among the preeclamptics who earned less than 10,000 per month. For calcium, both the cases and the controls with income levels less that 10,000 had higher mean serum levels, and it was statistically significant among the controls. This was not the case for the other micronutrients (see table 12).

Table 12: Comparison of mean serum levels of Vit. D, Se, Zn and Ca among the preeclamptics and normotensive earning less or more than 10,000 Kenya Shillings per month

Micronutrient	**Monthly income (cases)**		**P value**	**Monthly Income (controls)**		**P value**
	<10,000 (n=36)	≥10,000 (n=16)		<10,000 (n=29)	≥10, 000 n=25	
Vitamin D (ng/ml)	22.1±10.4	18.0±9.9	0.198	27.4±7.5	30.0±8.4	0.14
Selenium (mcg/l)	96.8±2.8	118.5±38.9	0.03	101±23.2	97.6±26.7	0.574
Zinc (μmol/l)	9.0±2.2	11.9±4.5	0.006	11.2±3.4	10.2±3.6	0.314
Calcium (mmol/l)	2.2±0.2	2.1±0.5	0.31	2.4±0.08	2.3±0.09	0.046

When focusing on age distribution and level of education, statistically significant differences were observed for selenium among the cases. The selenium levels were lowest amongst age group 21-30 and highest for those with a tertiary level of education (see table 13 and 14)

Table 13: Association of the serum levels of the select micronutrients and the age distribution among the preeclamptic and normotensive primigravida

Micronutrient	Age (Preeclamptics)			P value	Age (Normotensive)			P value
	≤20yrs n=9	21-30yrs n=37	>31yrs n=8		≤20yrs n=8	21-30yrs n=41	>31yrs n=5	
Vit. D (ng/ml)	24.9±11.8	19.3±9.9	23±9.1	0.276	27.4±9.5	29.4±7.8	24.1±5.7	0.14
Selenium (mcg/l)	114.8±25.6	95.6±30.2	127.3±42.5	0.024	90.5±17.0	102.2±26.5	93.2±16.5	0.574
Zinc (µmol/l)	9.6±3.3	9.8±3.8	11.3±3.7	0.538	9.6±3.7	10.7±3.6	12.4±1.3	0.314
Calcium (mmol/l)	2.0±0.7	2.26±0.17	2.27±0.08	0.168	2.36±0.06	2.33±0.1	2.27±0.07	0.046

Table 14: Association of the serum levels of the select micronutrients and the level of education among the preeclamptics and normotensive women

Micronutrient	Level of education (cases)			P value	Level of education (controls)			P value
	Primary n=4	**Secondary n=45**	**Tertiary n=5**		**Primary n=6**	**Secondary n=41**	**Tertiary n=7**	
Vit. D (ng/ml)	*17.8±10.5*	*21.1±10.4*	*19.9±9.5*	*0.81*	*34.8±9.2*	*27.6±7.8*	*29.2±5.6*	*0.115*
Selenium (mcg/l)	94.1±10.9	100.1±30.0	141.5±51.4	0.023	80.5±14.2	101.3±23.6	106.7±32.7	0.112
Zinc (µmol/l)	8.0±1.8	9.9±3.6	11.7±5.5	0.333	8.9±2.0	10.7±3.6	12.1±3.6	0.279
Calcium (mmol/l)	2.19±0.34	2.22±0.34	2.28±0.09	0.919	2.32±0.07	2.33±0.1	2.32±0.05	0.892

Note that in table 12, the selenium levels among the cases increase with increasing level of education. For vitamin D, though not statistically significant, the serum levels are much lower in the lower education status for the preeclamptics, while it is not so for the controls.

Pattern of consumption of specific nutrient rich foods for the different micronutrients among the preeclamptics and normotensive controls

The specific foods rich in vitamin D asked about in this study included: mushroom, fish and eggs. Frequent fish consumption was observed more among the controls, p-value 0.028 (Table 15). Frequent consumption in this study implied habitual daily or weekly intake. Only 20% of the entire study population ate mushroom at least once a month, with an almost half and half distribution between cases and controls. For foods rich in calcium, controls consumed milk, yoghurt and cheese more frequently compared to cases. These observations were statistically significant for consumption of yoghurt (p=0.014).

Regarding foods rich in selenium, it was noted that frequent beef consumption was more among the cases than the controls (p=0.014). Sunflower seeds, Brazilian nuts and green vegetables portrayed similar patterns between cases and controls. Brazilian nuts, which are considered the richest source of selenium was not popular amongst our study population, and only 7%,

consumed them in the past month. Green vegetables were well liked among our study subjects, 95% response on their frequent consumption. Green vegetables* are a good source of zinc, selenium and calcium. Kidney beans and peanuts, rich sources of zinc were consumed similarly when comparing cases and controls. Only 3% of the patients had consumed sunflower seeds, rich in zinc, in the past month.

Table 15: Proportion participants who consume specific foods rich in Vitamin D, calcium, selenium and zinc among the preeclamptics and the controls.

Consumption of specific vitamin D rich foods					
Food	**Consumption**	**Total (%)**	**Cases (%)**	**Controls (%)**	**P-Value**
Fish (n=106)	Frequent	65(61)	27(51)	38(72)	0.028
	Occasional/Never	41(39)	26(49)	15(28)	
Eggs (n=106)	Frequent	79(75)	39(74)	40(75)	0.823
	Occasional/Never	27(25)	14(26)	13(25)	
Mushroom (n=108)	Consumed in the past month	22(20)	10(19)	12(22)	0.632
	Never	86(80)	44(81)	42(78)	
Consumption of specific foods rich in *calcium					
Cheese (n=107)	Frequent	9 (8)	2(4)	7(13)	0.093
	Occasional/Never	98(92)	52(96)	46(87)	
Yoghurt (n=107)	Frequent	45(42)	16(30)	29(54)	0.014
	Occasional/Never	62(58)	37(70)	25(46)	
Milk (n=108)	Frequent	74(69)	33(61)	41(76)	0.097
	Occasional/Never	34(31)	21(39)	13(24)	
*Green vegetables (n=102)	Frequent	95(93)	49(96)	46(90)	0.436
	Occasional/Never	7(7)	2(4)	5(10)	
Consumption of specific foods rich in *selenium					
Beef (Grass-fed) n=105	Frequent	90(86)	49(94)	41(77)	0.014
	Occasional/Never	15(14)	3(6)	12(23)	
Sunflower seeds n=108	Consumed in the past month	6(6)	1(2)	5(9)	0.092
	Never	102(94)	53(98)	49(91)	
Brazilian nuts n=108	Consumed in the past month	8 (7)	6(11)	2(4)	0.141
	Never	100(93)	48(87)	52(96)	
Consumption of specific foods rich in *zinc					
Pumpkin seeds n=108	Consumed in the past month	3(3)	3(6)	0	-
	Never	105(97)	51(94)	54(100)	
Kidney beans n=108	Frequent	77(71)	39(72)	38(70)	0.832
	Occasional/Never	31(29)	15(28)	16(30)	
Peanuts n=107	Frequent	31(29)	12(23)	19(35)	0.153
	Occasional/Never	76(71)	41(77)	35(65)	

CHAPTER FOUR

DISCUSSION

The results obtained from this study showed that there are no demographic differences between the cases and the controls. Half (50%) of the cases had vitamin D deficiency compared to 13% of the controls, with the mean serum levels of Vitamin D being lower among the cases. Mean serum calcium was lower among the cases compared to the controls, while mean serum levels of selenium and zinc was the same between the cases and the controls. Severity of preeclampsia did not affect the mean serum levels of the micronutrients. Systolic and diastolic blood pressure correlated negatively with vitamin D levels but was unaffected by the other micronutrients. The level of consumption of specific nutrient rich foods rich in Vitamin D and calcium was more among the controls. These findings are going to be discussed below:

Demographic characteristics

The mean age for the patients with preeclampsia presented in the current study was 24.7±4.2 a majority, 72%, being between ages 20-30 years. This result correlates with what has been observed in other similar studies in Africa. In a teaching hospital in Nigeria for example, [101], presented a mean age of 27.4±4.9 years for the cases in their study, majority being in the age group 25-29. In Ethiopia, [102], found that most of the patients with preeclampsia, 87.86%, were in the age group 16-30 years. On the contrary, other authors have shown that preeclampsia is more likely to occur in women of advanced maternal age compared to younger women [98, 103].

Nonetheless, extremes of ages (<20 and > 40 years) are recognized risk factors for preeclampsia [104]. The mechanisms underlying these age related observations for preeclampsia are yet to be

expounded. In Africa however, more women are pregnant between ages 20-30 years, and this may partly explain why the average age of occurrence of preeclampsia falls in this group. The reason for advanced maternal age being a risk factor for preeclampsia could be related to the aging uterine vessels and subsequent defective placentation [98]

There was no association between income and preeclampsia even though those with an average income less than 10,000 per month were slightly more among the cases, 55%. Preeclampsia has been observed to occur more commonly among women of black ethnicity, part of the explanations being socioeconomic status as well as genetics [105] Low socioeconomic is an established risk factor for PE [46]. There was no observable correlation between level of education and preeclampsia in this study. This could be because over 90% (n=108) of the patients in the current study had attained at least secondary education and therefore relating it as a risk for preeclampsia was not feasible. Other studies nonetheless, done in low-income countries, have reported that low education status poses a risk factor for preeclampsia [39, 106].

Obstetric characteristics

In this study, majority of the patients with preeclampsia presented in the 3rd trimester (93.5%), the average gestational age being 35.2±4.4 weeks. This is an expected distribution since, 90% of preeclampsia occurs after 34 weeks gestation [1, 107]. Preeclampsia is classified as early onset or late onset preeclampsia depending on whether it occurs before or after 34 weeks gestation. Preeclampsia before 34 weeks is rare, and when it occurs, it tends to be severe [107]. Over half of the cases in our study population had severe preeclampsia, 61.1%, showing that severe preeclampsia is likely to be more common in our institution than preeclampsia without severe

features. Lifestyle choices and obesity have been implicated in the increasing incidence of severe preeclampsia in the United States over the years [108], which may be the case in our setup as well.

The mean systolic and diastolic blood pressures among the preeclamptics presented in this study is much higher than that documented by other authors. The blood pressure of the controls on the other were similar compared to that presented by the same authors. The SBP for the cases in the current study was 155.7±17.4mmHg while DBP was 123±11.1mmHg and SBP of the controls was 105±10.2 and DBP of 74.2±7.1. Akinloye et al [81] looking at a Nigerian population, presented a lower SBP of 144.3±3.9mmHg and DBP of 93.0±2.9mmHg among the cases, the controls had SBP of 110.4±6.4 and DBP of 73.3±6.8.

Jenkins et al. [109], presented similar values of SBP to those of the current study for Caucasians and African American women. The SBP of preeclamptic women was 157±16.1 and 160±18 for the Caucasians and African American women respectively. The DBP was 94±10.0 and 94±10.6 respectively, much lower compared to that of the present study. There is evidence that the clinical features of preeclampsia portray racial disparities, such that women of African ancestry have been shown to demonstrate more severe hypertension and to require aggressive antihypertensive treatment while Caucasian women are likely to have more of low platelets and hemolysis syndromes [110]. Genetic variations of the angiotensinogen gene are thought to be part of the explanation for these observations.

Micronutrient deficiency and comparison of mean serum levels between cases and controls as well as association with demographic, obstetric and nutritional parameters

Similar to the findings of other studies, Vitamin D deficiency was more likely to be present among women with preeclampsia than those who were normotensive ones with incidences of 50% and 13% respectively. Ringrose et al. [111], also found that 29% of the women with preeclampsia were deficient of vitamin D compared to 13% of the normotensive women. These women with preeclampsia were are also more likely to have low mean serum Vitamin D levels in comparison to normotensive ones. These findings are in agreement with other authors in other low and middle-income countries (LMIC) (95, 111, 15). Even though black race is an independent risk factor for vitamin D deficiency, with reported incidences of between 66-100% in black populations, countries with traditions of whole body covering due to religious reasons, such as India, Turkey and Iran, had lower means of Vitamin D than ours for both the preeclamptics and normotensive women (Table 16). Further, in Northwest Iran a study by Sadin et al. [112] found that none of the women in their study had sufficient levels of Vitamin D. These authors established that the percentage of deficiency and insufficiency of vitamin D was 60% and 40% respectively for women with preeclampsia compared to 10% and 90% for those who were normotensive controls.

Table 16: Serum levels of Vitamin D in different regions comparing preeclamptic and normotensive groups

Authors	Region	Serum Vit D in PE group (ng/ml)	Serum Vit D in Normotensive group (ng/ml)	P value
Singla et al. [95]	India	9.7 ± 4.95	14.8 ± 6.68	0.0001
Mohaghegh et al. [113]	Iran	15.2 ± 13.6	23.3 ± 15.3	0.001
Bukacak et al. [114]		19.3±4.31	23.7±5.93	0.001
Gupta et al. [15]	India	3.9	9	-
Current study	Kenya	20.8±10.2	28.6±7.9	<0.001

Several mechanisms have been postulated on how low vitamin D status would result in preeclampsia. First Vitamin D is thought to play a role in the synthesis and regulation of genes that are responsible for early placental development [115]. Second, Vitamin D has been thought to be a potent endocrine suppressor role in renin biosynthesis for the regulation of the renin-angiotensin system (RAS), an important regulator of fluid metabolism [114]. Thirdly, it plays a role in placental immunomodulation, and is thought to have anti-inflammatory properties [116]. The relationship between vitamin D and preeclampsia is complex, some authors have pointed out that low levels of this micronutrient in the second trimester may be an indicator of preeclampsia [92].

Whether to supplement vitamin D in pregnancy is still a puzzle. A recent met analysis of 15 trials by De-Regil et al. [117] showed that supplementation of Vitamin D by a single or continued dose, may increase serum levels with resultant benefits of risk reduction for preeclampsia, low birth weight and preterm birth. Whether this can be implemented, as part of routine antenatal care requires policy changes for specific regions. RCOG [99] recommends routine

supplementation of Vitamin D in pregnancy particularly for those at risk, such as those with dark skin, hidden from the sun, obese or socially excluded.

Although other authors have established that mean serum vitamin D level would be lower in women with severe preeclampsia in contrast to those with preeclampsia without severe features [114], this was not the case for our study. However, it was noticed that serum vitamin D correlated negatively with SBP and DBP, such that the lower the blood pressure, the higher the levels of vitamin D and vice versa. This observation is similar to what was presented by Umar et al. [118] who also found an inverse relationship of Vitamin D and blood pressure. There is weak evidence to support that Vitamin D supplementation in non-pregnant adults with hypertension results in a reduction of blood pressure but this was not the case for normotensive ones [119].

Patients with preeclampsia had lower levels of serum calcium in this study. Other studies done in LMIC have presented similar findings [18, 69, 67, 73, 66, 68]. Notably, countries in Africa and India presented much lower levels of serum calcium in both cases and controls. One of the mechanisms in which calcium levels affects blood pressure is that, low calcium results in an increase of intracellular calcium including within vascular smooth muscle cells, which then causes vasoconstriction and eventually a rise in SBP and DBP [61]. Low calcium levels also result in a rise in parathyroid hormone and renin, which in turn cause a rise in blood pressure [61, 120].

The World Health Organization (WHO) proposed supplementing calcium for women in regions that are calcium deficient [121]. A meta- analysis done in developing countries, which looked at

10 randomized control trials, established that calcium supplementation during pregnancy is associated with a reduction of risk for gestational hypertension, pre-eclampsia, neonatal mortality and preterm birth and no notable side effects on the women [122]. Encouraging dietary intake of foods rich in calcium maybe another alternative so as to reduce pill load, however this is largely dependent on the patient's ability to deliberately consume calcium-rich foods. This study established nutritional habits relate to the status of serum calcium in our setup, since consumption of dairy products with high calcium content such as yoghurt and cheese as well as milk was more among the normotensive controls whose mean calcium levels were much higher.

Even though other studies have found that low serum selenium and zinc occur in women with preeclampsia, this was not the case in our study. Ghaemi et al. [87], and Farzin and Sajadi [67] studying Iranian populations presented significantly lower levels of Se in the PE group. Among Nigerian patients, Akinloye et al. [81] also presented lower levels in the PE groups. Serum Zinc levels has also been established to be lower among women with preeclampsia in various studies [81, 21, 67, 68]. This differences in serum levels of zinc and selenium between populations is likely to be influenced by dietary patterns.

Consumption of specific nutrient rich foods for zinc and selenium was optimal for both the women with preeclampsia and those who are normotensive for participants of the current study, specifically beef and green vegetables. It was also noted that serum levels of selenium were higher with increasing level of education and level of income. A possible explanation is the frequent consumption of beef (grass-fed) by the study population, which is thought to relate to level of income. If women in our setup continue with the dietary patterns depicted in this study,

the serum levels of these micronutrients will remain relatively stable. The findings of this study do not negate the association of zinc and selenium with preeclampsia but we do acknowledge that these micronutrients may not be implicated in the disease process for the women in our study population. These findings emphasize the need for region specific surveys for micronutrient deficiencies.

CONCLUSION

Patients with preeclampsia exhibited low levels of calcium and Vitamin D in comparison to their normotensive counterparts. Certainly, the proportion of study participants with vitamin D deficiency was high among those with preeclampsia. These women were also less likely to consume specific foods rich in calcium and vitamin D. Contrary to what has been presented in other populations, serum levels of zinc and selenium did not show any association with preeclampsia

RECOMMENDATIONS

Calcium and Vitamin D supplementation in pregnancy for women thought to be deficient in these micronutrients is recommended. Supplementation of calcium and Vitamin D in women who are at risk of preeclampsia can also be done, because the findings of this study have established low levels of Ca and Vit. D is an additional risk to PE development. In view of the dietary patterns presented in this study, pregnant women should be encouraged to be deliberately on consume locally available foods rich in calcium and vitamin D, such as milk, natural yoghurt, eggs and green vegetables.

This study also provides a basis for conducting a randomized control trial focusing on supplementation of vitamin D and calcium individually or combined and assessment of these interventions on preeclampsia prevention for our setup.

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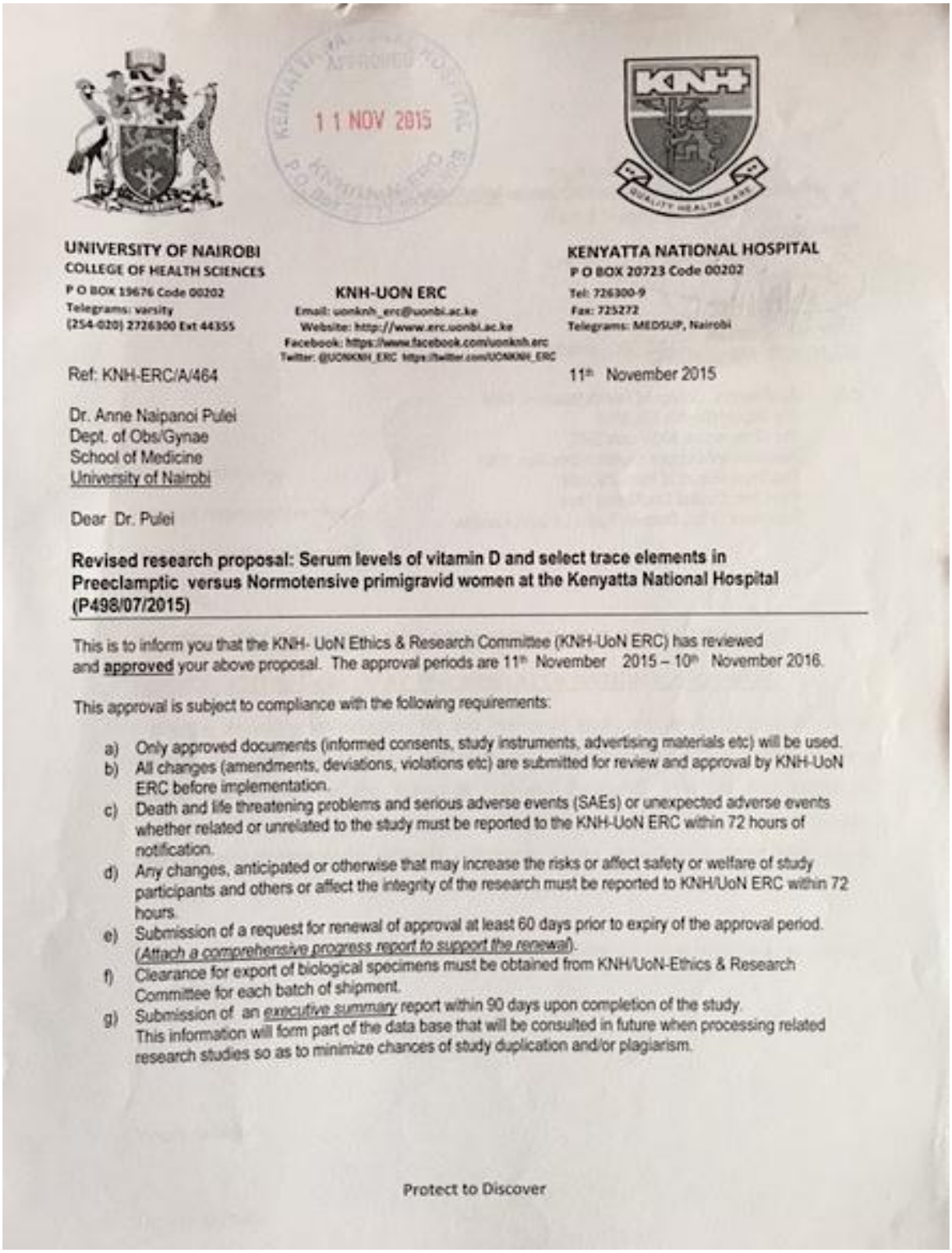
APPENDIX 1: KNH/UON-ERC APPROVAL LETTER



1 1 NOV 2015

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11th November 2015

Dr. Anne Naipanoi Pulei
Dept. of Obs/Gynae
School of Medicine
University of Nairobi

Dear Dr. Pulei

Revised research proposal: Serum levels of vitamin D and select trace elements in Preeclamptic versus Normotensive primigravid women at the Kenyatta National Hospital (P498/07/2015)

This is to inform you that the KNH- UoN Ethics & Research Committee (KNH-UoN ERC) has reviewed and **approved** your above proposal. The approval periods are 11th November 2015 – 10th November 2016.

This approval is subject to compliance with the following requirements:

a) Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b) All changes (amendments, deviations, violations etc) are submitted for review and approval by KNH-UoN ERC before implementation.
c) Death and life threatening problems and serious adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH-UoN ERC within 72 hours of notification.
d) Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH/UoN ERC within 72 hours.
e) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (*Attach a comprehensive progress report to support the renewal*).
f) Clearance for export of biological specimens must be obtained from KNH/UoN-Ethics & Research Committee for each batch of shipment.
g) Submission of an *executive summary* report within 90 days upon completion of the study. This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/or plagiarism.

Protect to Discover

APPENDIX 2: DATA COLLECTION SHEET/QUESTIONNAIRE

DEMOGRAPHIC DATA

1. Number----------
2. Phone Number ----------
3. Initial----------
4. Age----------
5. Religion----------
6. Residence in the past year----------
7. Occupation----------
8. Average income per month----------
9. Marital Status----------
10. Education Level (circle one)
    a. None/ Early childhood education
    b. Primary
    c. Lower secondary
    d. Upper Secondary
    e. Post secondary non tertiary
    f. Tertiary (Bachelors)
    g. Tertiary (Masters)
    h. Tertiary (Doctoral)]

A. OBSTETRIC CHARACTERISTIC

a. Gestational Age----------

B. CLINICAL PARAMETERS (IF NORMOTENSIVE FILL 1 ONLY)

1. Blood pressure on admission
    a. Systolic----------
    b. Diastolic ----------
2. Proteinuria ----------

3. Presence of symptoms (CIRCLE)
    a. Headache
    b. Epigastric pain
    c. Blurred Vision
4. Liver function tests on admission
    a. ALT
    b. AST
    c. Albumin
5. LDH
6. Haemogram on admission
    a. Platelets
    b. WBC
    c. RBC
7. Presence of seizures---------- (indicate YES OR NO)

C. SERUM BIOCHEMICAL LEVELS

1. Vitamin D----------
2. Selenium----------
3. Zinc----------
4. Calcium----------
5. Magnesium ----------

D. DIETARY ASSESSMENT

1. Height----------
2. Weight----------
3. Body Mass index----------
4. How often do you take servings of fish (any Tuna, Tilapia, Nile perch, Omena, Red snapper): circle one
    a. Daily, indicate how many times a day----------

b. Weekly, indicate how many times a week----------
c. Monthly, how many times a month----------
d. Once in a while----------
e. Never, if so, state the reason why----------

5. How often do you consume the following dairy products
    a. Milk (circle one)
        i. Daily, indicate how many times a day----------
        ii. Weekly, indicate how many times a week----------
        iii. Monthly, how many times a month----------
        iv. Once in a while----------
        v. Never, if so, state the reason why----------
    b. Yoghurt
        i. Daily, indicate how many times a day----------
        ii. Weekly, indicate how many times a week----------
        iii. Monthly, how many times a month----------
        iv. Once in a while----------
        v. Never, if so, state the reason why----------
    c. Cheese
        i. Daily, indicate how many times a day----------
        ii. Weekly, indicate how many times a week----------
        iii. Monthly, how many times a month----------
        iv. Once in a while----------
        v. Never, if so, state the reason why----------
6. How often do you eat Green Vegetables (any: spinach, kale, managu, kunde)
        i. Daily, indicate how many times a day----------
        ii. Weekly, indicate how many times a week----------
        iii. Monthly, how many times a month----------
        iv. Once in a while----------
        v. Never, if so, state the reason why----------
7. How often do you eat nuts (Peanuts, cashews, almonds)
        i. Daily, indicate how many times a day----------

ii. Weekly, indicate how many times a week----------
iii. Monthly, how many times a month----------
iv. Once in a while----------
v. Never, if so, state the reason why----------

8. How often do you have fruits
   i. Daily, indicate how many times a day----------
   ii. Which fruits do you consume frequently----------

   ---
   iii. Weekly, indicate how many times a week----------
   iv. Monthly, how many times a month----------
   v. Once in a while----------
   vi. Never, if so, state the reason why----------
9. How many times do you eat meat (Beef, pork, chicken)
   i. Daily, indicate how many times a day----------
   ii. Weekly, indicate how many times a week----------
   iii. Monthly, how many times a month----------
   iv. Once in a while----------
   v. Never, if so, state the reason why----------
10. How often do you eat beans
   i. Daily, indicate how many times a day----------
   ii. Weekly, indicate how many times a week----------
   iii. Monthly, how many times a month----------
   iv. Once in a while----------
   v. Never, if so, state the reason why----------
11. How often do you take Brown bread?
   i. Daily, indicate how many times a day----------
   ii. Weekly, indicate how many times a week----------
   iii. Monthly, how many times a month----------
   iv. Once in a while----------
   v. Never, if so, state the reason why----------

12. Have you ever taken the following foods? Drinks?

a. Roibos tea
    i. No----------
    ii. Yes------- (How many times in the past month---------)
b. Pumpkin seeds
    i. No---------
    ii. Yes-------- (How many times in the past month----------)
c. Mushroom
    i. No----------
    ii. Yes----------(How many times in the past month---------)
d. Sunflower seeds
    i. No----------
    ii. Yes----------(How many times in the past month----------)

e. Brazilian nuts
    i. No----------
    ii. Yes----------(How many times in the past month--------)
f. Brown rice
    i. No----------
    ii. Yes----------(How many times in the past month-------)

13. In your opinion which food do you eat most frequently (Include combinations if need be) --
---

APPENDIX 3: CONSENT FORM IN ENGLISH

CONSENT FORM

Title of the study

Serum Levels of Vitamin D and Select Trace Elements in Preeclamptic and Normotensive Primigravid Women at the Kenyatta National Hospital

Introduction

I am Dr. Anne Naipanoi Pulei, interested in carrying out a research on preeclampsia. Preeclampsia is a disease that is specific to pregnant women. A woman with this disease gets high blood pressure and damaged organs such as the kidney and liver. Such a patient may be seen to have yellow eyes, swelling of the legs, face and sometimes the whole body. When this disease becomes worse, the patient may even fail to see, bleed in the brain and sometimes a convulsion, which we call eclampsia. The disease also affects growth of the baby and sometimes the baby dies in the womb. This illness is common in our setup, therefore research on it would be of value to try and prevent it. Levels of certain minerals we find in some foods, when low, have been shown to be a risk factor for it. I would therefore check blood levels of these minerals in our population. When you agree to participate in the study, I will help you fill a questionnaire, then obtain blood samples from you to help us do this study. It is not a must that you participate in this study.

Objectives of the study

1. To check the blood levels of certain minerals in patients with preeclampsia and those without.

2. To check whether the blood levels of these minerals are associated with known risk factors (diet, occupation and level of education), severity of disease and gestational age in the different studied groups.

Humble request and voluntarism:

_____ In order to carry out this study, we need to obtain a blood sample from you to check the levels of these minerals. We will also ask you some questions such as how you eat, where you live, to enable us understand these deficiencies. Denial of consent will be duly respected. It is our wish that you participate in study as a volunteer after having understood our description above.

Procedures

We will talk to you first as mentioned above and fill a questionnaire with you. We'll then obtain blood from you left arm, about 5mls. This blood will be taken to the laboratory to check the minerals. We shall not carry any other test on this blood.

Benefits

The findings of this study may help us prevent the occurrence of this disease. In the event that we find low levels of these minerals in your blood, we'll first advice you on dietary practices that will improve these levels as well as increasing your exposure to sunlight and also prescribe appropriate supplements for you.

Risks

We do not anticipate any risks when you offer yourself to participate in this study. Safety measures will be taken when obtaining the blood samples.

Confidentiality:

The name of the subject will not appear either on the data sheets or in the final thesis.

Incase of further explanations or inquiries, contact me, the principal investigator, Dr. Anne Naipanoi Pulei on 0722465924 or by email anmunkush@yahoo.com. You can also contact the Kenyatta National Hospital/University of Nairobi-Ethics Review Committee (UoN/KNH ERC) on (254-020) 2726300 Ext 44355

I, the undersigned have been explained to and understood the above and willingly accept to participate in the study.

Signature / Thumbprint: _____ Date:_____

I, the investigator having explained in detail the purpose for the study, hereby submit that privacy of the data recorded shall be maintained and no details will be revealed, apart from those related to the study.

_____

Signature: ____Date:_____

APPENDIX 4: CONSENT FORM IN KISWAHILI

CHETI CHA RUHUSA

MADA

Uchunguzi wa madini wa damu na Vitamini D kwa wagonjwa walio na shinikizo la damu wakiwa wajawazito

Utangulizi

Mimi naitwa Dr. Anne Naipanoi Pulei, ningependa kufanya utafiti juu ya ugonjwa wa shinikizo la damu kwa wajawazito ambao unaitwa kwa kimombo “preeclampsia”. Ugonjwa huu hupata akina mama walio na mimba haswa na husababisha mishipa kuharibika na unaweza kufanya mama kuwa na kifafa na hata kuharibu ukuaji wa mtoto. Ugonjwa huu unaendelea kuongezeka hapa kwetu. Kuna madini ambazo huwa tunapata kwenye chakula, zikiwa chini kwa damu, huweza kusababisha ugonjwa huu. Lengo la uchunguzi huu ni kuangalia kama kuna tofauti kwa damu, wagonjwa walio naona wale hawana. Ukiwa utakubali kuhusika na uchunguzi huu, tutakupima damu. Sio lazima kukubali

Lengo la Uchunguzi

3. Kuchunguza kadiri za madini fulani pamoja na vitamin D kwenye damu ya wagonjwa
4. Kuchunguza kama kipimo cha damu vya madini vinaambatana na jinsi mtu anakula, anapoishi na pia ukali wa ugonjwa

Manufaa

Manufaa ya uchunguzi huu ni kwamba, ikiwa itapatikana kuwa kuna uhusiano wa madini haya kuwa chini kwenye damu na matokeo ya ugonjwa huu wa “preeclampsia” au shinikizo la damu, tutachukulia hatua ili tuweza kupunguza ugonjwa huu. Tukipata kuwa damu yako ina upungufu wa madini haya tutakuelezea ule vyakula ambavyo vina madini haya kwa

wingi na pia tutakuandikia madawa yanayoweza kuyaongeza kwenye damu na kukueleza uote jua kwa masaa fulani ikiwa Vitamini D ndiyo ilipungua.

Uhusika wa hiari.

Kukubali kusaidia katika uchunguzi huu si lazima na tena hamna gharama yoyote na pia tutaelewa ikiwa hutaweza

Usiri

Jina la mgonjwa halitatumiwa mahali popote katika uchunghuzi huu wala kwa kwa matokeo yatakayochapishwa.

Mimi nimekubali kuwa nitasaidia katika uchunguzi huu baada ya kuelezewa lengo na manufaa ya uchunguzi huu.

Sahihi _____

Mimi mchunguzi nimemweleza kuhusu uchunguzi huu naapa kutimiza usiri wa matokeo yote ya uchunguzi huu.

Sahihi _____

APPENDIX 5: BUDGET AND FUNDING

Determination of serum levels of the trace elements and vitamin D was carried out in a private laboratory for uniformity and quality control. The cost incurred is as listed in the table below. Some of the assays listed were more expensive because they are not routine assays and were transported out of the country for analysis. The study was partially funded by the student and a private laboratory.

Table 17: Budget of the study

ITEM	ITEM NUMBER	UNIT COST (Ksh)	TOTAL
Serum vitamin D	108	4,299	464,292
Serum selenium	108	3,399	367,092
Serum calcium (including Albumin)	108	1,498	161,784
Serum zinc	108	4,499	485,892
Serum magnesium	108	699	75,492
Research Assistants	1	25,500	25,500
Statistician	1	35,000	35,000
TOTAL			**1,615,052**

APPENDIX 6: WORKPLAN

The timelines in which the study was carried out is as indicated in the table below.

Table 18: Study schedule

STEPS IN THE RESEARCH PLAN	COMPLETION
Presentation of the proposal to the department	July 2015
Submission of proposal to ethics	August 2015
Re-Submission of the proposal to ethics	October 2015
Ethical approval	November 2015
Training of Research Assistants, setting up logistics of data collection	January-February 2016
Data collection	March 2016-June 2016
Data Analysis	July 2016
Compilation of results	August 2016
Presentation of final research to the department	October 2016
Submission of the thesis to the department	November 2016