Relationship between Serum Zinc Levels and Preeclampsia in Pregnant Women at the University Teaching Hospital, Lusaka, Zambia

By

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A dissertation submitted to the University of Zambia in partial fulfillment of the requirements of the Degree of Master of Science in Biochemistry

THE UNIVERSITY OF ZAMBIA
LUSAKA

2016

DECLARATION

This dissertation is the original work of **CHABABA LIKANDO**. It has been done in accordance with the guidelines for M.Sc dissertations for the University of Zambia. It has not been submitted elsewhere for a degree at this or another University.

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I, **CHABABA LIKANDO**, do hereby certify that this dissertation is the product of my own work and, in submitting it for my Master of Science in Biochemistry program, further attest that it has not been submitted to another University in part or whole for the award of any program.

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Preeclampsia is one of the leading causes of maternal mortality and pre-term delivery around the world. Though its exact cause is not yet known, it is found more commonly in developing countries. Deficiency of serum Zinc has been implicated in the pathophysiology of preeclampsia due to its crucial role as a cofactor of antioxidant enzymes as oxidative stress is a hallmark of preeclampsia. Reports from various regions of the world are controversial and largely inconclusive. In Zambia, the relationship between serum Zinc levels and preeclampsia remains unexplored yet Zambia lies within the geographical region thought to be most deficient in Zinc**.** The purpose of the study was to investigate the association between serum Zinc levels and preeclampsia in pregnant women at University Teaching Hospital, Lusaka, Zambia.

A comparative cross-sectional study design was employed on purposively sampled 41 preeclamptic and 57 non-preeclamptic pregnant women over a four month period from February 2016 to May 2016 at the maternity clinic of the University Teaching Hospital. Systolic blood pressure > 140 mm Hg and/ or diastolic blood pressure > 90 mm Hg with proteinuria atleast 1+ defined a preeclamptic case. Control participants comprised healthy pregnant women who were attending antenatal services. An interview guide was used to collect data on social demographics. Serum samples from participants were analysed for Zinc concentration by Atomic Absorption Spectrophotometry. Data was analysed using Student T-test and entered in Stata version 14.

Maternal age was identified as a risk factor of preeclampsia in that there was significantly greater proportion of preeclamptic women aged between 30 and 40 years (61%; $p = 0.006$) than that of preeclamptic women aged between 18 to 30 years of age (32%; $p = 0.018$). However, there was no significant difference in the mean serum Zinc levels of the preeclamptic (89.17 ± 47.19 µg/dL) versus the non-preeclamptic pregnant women (76.20 ± 35.23 µg/dL) ($p = 0.122$). The data also showed that maternal age group, gestational age, family history of preeclampsia, and residential area density had no significant effect on the relationship p between serum Zinc level and preeclampsia (p values > 0.05). No correlation was found between blood pressure and serum Zinc levels in the total sample (SBP: $r = 0.149$, $p = 0.2599$; DBP: $r = 0.1656$, $p = 0.1031$) or in the preeclamptic cases alone (SBP: $r = -0.0636$, $p = 0.6928$; DBP: $r = 0.0452$, $p = 0.7788$).

Our results implied lack of a direct relationship between serum Zinc level and preeclampsia in the pregnant women at UTH. Incidentally, maternal age was found to be a risk factor for preeclampsia. Serum Zinc is of doubtful clinical value in preeclampsia. Nonetheless, further investigation in the role of serum Zinc and other minerals/ metabolites in preeclampsia is merited as they may act as early predictors of the condition.

Key words: preeclampsia, Zinc level, pregnant women, University Teaching Hospital.

*To my mother, **Loureen Mwangala Chababa**, for her inspiration, love and support during the period of this study*

ACKNOWLEDGEMENTS

I would like to thank my supervisors Dr. G. Sijumbila (Physician, Biochemist, Head of department – Physiological Sciences, University of Zambia), Prof. B. Vwalika (Physician, Obstetrics and Gynecology specialist, Epidemiologist, Head of department – Obstetrics and Gynecology, University Teaching Hospital, Lusaka), and Mr. M. Mukosha (Pharmacist, Biochemist – University of Zambia) for their untiring guidance and support in the development of the study proposal and this dissertation.

I am also highly indebted to Dr. M. Maimbolwa and NORHED for the financial and moral support of this project.

Additionally, I would like to acknowledge Mr. A. Banda for help in the data analysis, Mr. M. Sakala (Lab manager at Food and Drugs Control Laboratory, Ministry of Health) for the laboratory analysis, Mr. P. Musongole and Monde (Midwives at University Teaching Hospital Maternity Clinic) and Mrs. Mundia (Matron in Charge) for the assistance in data collection, and everyone else not named here including family and friends who have been supportive in this project.

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LIST OF ACRONYMS

1. UNZA – University of Zambia
2. UTH – University Teaching Hospital
3. SBP – Systolic Blood Pressure
4. DBP – Diastolic Blood Pressure
5. SOD – Superoxide Dismutase
6. NOS – Nitric Oxide Synthase
7. PE – Preeclampsia
8. ROS – Reactive Oxygen Species
9. WHO – World Health Organization
10. MOH – Ministry of Health
11. AAS – Atomic Absorption Spectrometry
12. Ppb – Parts per billion
13. NIST - National Institute of Science and Technology

GLOSSARY OF TERMS

1. **Proteinuria**: Presence of protein in urine
2. **Hypertension**: A condition of elevated systolic blood pressure above 140 mm Hg and/ or elevated diastolic blood pressure above 90 mm Hg
3. **Participants**: Persons who enlist to be part of a study project
4. **Preeclampsia**: A multisystem pregnancy disorder characterized by new onset of hypertension after 20 weeks of pregnancy with proteinuria
5. **Gestational age**: Age of the pregnancy in weeks

CHAPTER ONE

BACKGROUND AND INTRODUCTION

1.1 Introduction

Preeclampsia is a multisystem disorder characterized by the new onset of hypertension (blood pressure >140/90 mm Hg) often accompanied by proteinuria in the second half of pregnancy (Fukui et al., 2012). It can be deleterious to both maternal and child health as it leads to premature and risky labor and in extreme cases causes end organ damage. It is estimated globally that 5-7 percent of all pregnancies are affected by preeclampsia and it is "the most common yet least understood disorder of pregnancy" (Akinloye et al., 2013).

While cases of preeclampsia are found worldwide, they are more prevalent in developing countries of Africa. It is not known for certain why this is so, but some evidence points to genetic susceptibility being one of the factors since preeclampsia is also more common in black communities in other continents apart from Africa (Jain et al., 2010). Additionally, research has shown that micronutrient malnutrition is more prevalent in African countries than other regions and this could be a predisposing factor to numerous disease conditions (Gibson, 2012).

Though its exact etiology remains unknown, preeclampsia has been found to be associated with many risk factors. These include family history, new paternity, multiple gestation, nulliparity, maternal age (<20 years or >35 years), pre-existing hypertension or diabetes, and being of the black race (Callahan et al., 2007). In addition, micronutrient deficiencies have been thought to be associated with either the onset or progress of the disease. Micronutrients include minerals, vitamins, and other molecules needed by the body in relatively small quantities and have critical roles to play in physiologic systems as intermediates or enzyme cofactors. In pregnancy, physiologic redistribution of mineral elements to nourish the baby can lead to maternal deficiencies if the nutritional supply is inadequate (Mori et al., 2012).

Zinc is important in the maintenance of the vascular tone in blood vessels. As an antioxidant mineral, it is a component of various protein enzymes such as superoxide dismutase (SOD) and nitric oxide synthase (NOS) which are meant to protect the integrity and tone of the endothelium smooth muscle from destructive effects of reactive oxygen species (ROS). In preeclampsia, vascular oxidative stress is increased as shown by various studies (Hovdenak and Haram, 2012). Deficiency in serum Zinc could therefore be a mediatory factor in weakening of the indigenous antioxidant defense systems because without the metal cofactor the enzymes do not operate at optimum, and in this way oxidative stress is linked with the hypertension in preeclampsia (Palei et al., 2013).

Investigations of the Zinc status of pregnant women in Africa are limited, but there is increasing evidence that they may be especially vulnerable to Zinc deficiency due to impoverished nutrition. Previous studies of pregnant women in various African countries, including Nigeria, Egypt, Congo DR, and Malawi, have all reported lower plasma Zinc concentrations compared to pregnant women from developed countries (Gibson, 2012).

Evidence seems to point to the fact that decreased serum levels of Zinc and other trace minerals are associated with the risk of preeclampsia in pregnant women, though there is still need of further research in various populations to establish the external validity of various findings (Akinloye et al., 2013, Kim et al., 2012, Rayman et al., 2003). But the subject is yet wrapped in obscurity as other researchers have brought forward contrary findings and have ruled out serum Zinc from having any role to play in the onset or progress of preeclampsia (Sibai, 1998).

Currently no studies have been done to investigate association between serum Zinc levels and preeclampsia in pregnant women in the Zambian population. Being the serious condition it is, knowledge of factors that associate with preeclampsia can be of help in the management of the disease. This research was therefore necessary in order to come up with local findings

within the Zambian population as to whether serum Zinc levels are associated with preeclampsia in pregnant women at the University Teaching Hospital (UTH).

1.2 Statement of the problem

According to MOH, preeclampsia is a leading cause of complications in pregnancies in Zambia. Currently, it is estimated that 591 women in every 100,000 live births die of complications mainly due to preeclampsia in Zambia (MOH, 2013).

In spite of relentless research efforts in various global regions and settings, the exact etiology of preeclampsia has not yet been fully elucidated.

Previous research has shown decreased Zinc levels to be linked with preeclampsia, and that pregnant women in developing countries like Zambia could be at a higher risk of being deficient in this mineral (Gibson, 2012). There is little or no evidence based information on the role of Zinc in preeclampsia in Southern Africa region, and Zambia in particular.

In an effort to address the problem, this study investigated the association of serum Zinc with preeclampsia in women selected for the study at the Maternity Clinic of the University Teaching Hospital, Lusaka, Zambia.

1.3 Justification of the study

Currently, little or no information has been documented on the role of Zinc in preeclampsia in pregnant women in Zambia. This study is therefore necessary for the purpose of establishing baseline data on the extent to which Zinc could be associated with preeclampsia in pregnant Zambian women.

Information generated by this study will be useful to health policy makers on the management of complications of pregnancy in Zambia. Furthermore, this research will add to the current existing scientific knowledge on the risk factors associated with preeclampsia and possibly open up major cohort research studies in the same field.

1.4 Main objective

To investigate the relationship between serum Zinc levels and preeclampsia in pregnant women attending maternity clinic at the University Teaching Hospital (UTH)

1.5 Specific objectives

1. To determine mean serum Zinc levels in preeclamptic and non-preeclamptic pregnant women attending maternity clinic at UTH
2. To determine the relationship between serum Zinc levels and blood pressure in preeclamptic and/or non-preeclamptic pregnant women attending maternity clinic at UTH
3. To determine the social demographic variables that are risk factors of preeclampsia

1.6 Research questions

1. Are Zinc levels low in women presenting with preeclampsia compared to normotensive pregnant women at the University Teaching Hospital?
2. Is there a linear relationship between serum Zinc level and blood pressure in preeclamptic and non-preeclamptic women?
3. What demographic variables are risk factors of preeclampsia?

CHAPTER TWO

LITERATURE REVIEW

Hypertensive disorders in pregnancy have been reported as the leading cause of complications of pregnancy globally and yet the least understood (Akinloye et al., 2013). Among the various types of hypertensive disorders of pregnancy, preeclampsia is the major one. As alluded to earlier, the exact etiology of preeclampsia has not yet been fully elucidated. The multitude of inconclusive theories put forward in an attempt to explain its origin and on-set has led to its being dubbed "the disease of theories" (Amirabi et al., 2015).

According to the Ministry of Health in Zambia, preeclampsia continues to be a leading cause of morbidity and mortality for both pregnant women and their unborn babies (MOH, 2013). Further, previous studies have associated the occurrence of preeclampsia in pregnancy with varied factors and the search continues for associated risk factors in an effort to understand its pathophysiology more thoroughly (Bahadoran et al., 2010).

It has been suggested that levels of certain trace elements such as Zinc and Selenium, due to their role in combating oxidative stress, might play an important role in preeclampsia (Rayman et al., 2003). Zinc is an important stabilizing component of antioxidant enzymes such as superoxide dismutase (SOD) and nitric oxide synthase (NOS), thus it is involved in the degradation of the harmful products of oxidative stress such as superoxide, hydroxide radicals, and peroxynitrite. It is believed that the powerful oxidizing and nitrating agent peroxynitrite is a candidate mediator of the endothelial dysfunction present in preeclampsia through interaction with signal transduction pathways linked to vasoactive agents (Rayman et al., 2003). Reports confirm that levels of peroxynitrite in the vasculature of preeclamptic women are significantly greater than in normal pregnancy (Roggensack et al., 1999). Zinc deficiency, therefore, has the capacity to indirectly invoke pathological changes that culminate in endothelial cell dysfunction (Palei et al, 2013).

Studies that have analyzed the role of Zinc with hypertension in general are numerous. There seems to be some consensus from both experimental and observational studies that deficiency in Zinc is associated with increase in blood pressure (Akinloye et al., 2013, Kim, 2013, Sato et al., 2002). But one of the studies reviewed has an opposing view and experimentally found that elevated Zinc intake increased the systolic blood pressure of normotensive rats (Yanagisawa et al., 2004).

In studies that deal with levels of Zinc in preeclampsia and associated risk factors, a few researchers came up with noteworthy findings. Coyle et al. (2013) showed that low Zinc levels are associated with hypertensive disorders in pregnancy, and that smoking and alcohol consumption aggravate the unavailability of Zinc to the body in spite of adequate dietary intake. Alcohol is believed to reduce renal reabsorption of Zinc while smoking causes serum Zinc to be unavailable to the tissues. In this way, smoking and alcohol drinking may be linked to incidence of preeclampsia. In our study smoking and alcohol consumption was not looked at as these are highly uncommon practices among pregnant women locally. But we went further to look demographic characteristics and assess their association with preeclampsia.

A cross-sectional study of Korean pregnant women also showed that serum levels of Zinc, Calcium and Iron are associated with the risk of preeclampsia. The results of this study suggested a strong association, though small sample sizes were used hence their study was quite vulnerable to random error (Kim et al., 2012). However, in a similar study but with larger sample sizes, Atomic Absorption Spectrometry (AAS) was used to assess the serum of 60 preeclamptic and 60 normal pregnant women. Their results showed significantly lower levels of Zinc, Selenium, Calcium, and Magnesium in the cases compared with controls (Farzin and Sajadi, 2012). In our study a similar method of determining zinc was used on purposively sampled 41 cases and 57 controls.

Studies by Gibson (2012) in Malawi explained that diets prevalent in unrefined cereals and legumes could be a risk factor for mild Zinc deficiency since the high content of phytic acid prevents the efficient absorption of Zinc and other divalent ions. It was actually found that the Zinc level in Malawian women who participated in the study who subsisted on such a diet also had suboptimal Zinc status (Gibson, 2012). The similarity in socio-economic and socio-cultural practice between Malawi and Zambia could help explain the reported Zinc deficiency in both countries.

Pregnancy itself is a condition of physiological and anatomical changes that has potential to cause decrease in maternal mineral levels in certain conditions (Brown et al., 2001a). The additional tissues that are used to develop the fetus and placental tissues demand for extra Zinc from the maternal blood system. Certain physiologic adjustments such as reduction in urinary and fecal loss of Zinc, increase in intestinal Zinc absorption, and release of maternal tissue Zinc into the blood stream help to maintain Zinc homeostasis to cater for the extra need for the mineral in pregnancy. However, it is reported that those mechanisms can be over-ridden in conditions of dietary insufficiency, hence predisposing the mother to conditions of Zinc deficiency (Brown et al., 2001b, Boskabadi et al., 2012).

Gibson (2006) concurs with and reinforces the idea that mineral nutrients, in particular Zinc, Iron, and Selenium have a role to play in pathogenesis of Hypertensive disorders in pregnancy. They report that in most poor countries in Africa, levels of those essential minerals have continued to be below normal in a large proportion of pregnant women hence predisposing them to risk factors for disorders such as hypertension (Gibson, 2006). Another study asserted that preeclampsia is related with oxidative stress and endothelial dysfunction, and thus, it is expected that lower serum levels of antioxidant nutrients such as Zinc would be found in preeclamptic women (Kim et al., 2012).

Most of the literature reviewed presents hope in the improvement of maternal health through supplementation of essential minerals to pregnant women. In this vein, Adam et al. (2001a) observed an increase in incidence of preeclampsia in certain Zinc deficient regions, and later it was found that Zinc-supplementation reduced the high incidence of the disease (Adam et al., 2001a).

On the contrary, some literature has reported contradictory findings and even expressed despair in that venture as far as prevention of preeclampsia is concerned (Caughey et al., 2005, Sibai, 1998). Prominent among the studies with contrary findings is the research that was done by Adam et al., (2001) investigating levels of plasma Zinc in preeclampic and non-preeclamptic participants. They reported that there was found no significant difference in the levels of the mineral in both comparison groups (Adam et al., 2001b).

In the same vein, Atamer et al. found that the difference between serum Zinc levels in their study and control groups was very insignificant (Bahadoran et al., 2010). Nevertheless, assays of trace elements, such as Zinc, which are linked with incidence of hypertensive disorders, still present hope of being used for early diagnosis of preeclamptic conditions (Kumru et al., 2003).

There has been considerable debate as to what biomarker is most reliable and sensitive as an indicator of Zinc status in individuals and populations. A variety of methods of Zinc assessment have thus been proposed and critiqued. In a detailed review by Lowe et al. (2009), 32 potential biomarkers from 46 publications were analyzed. It was concluded that plasma (or serum) Zinc, hair Zinc, and urinary Zinc were reliable enough as biomarkers of Zinc status in representative groups of populations as they also responded to dietary Zinc manipulations. To the contrary, platelet, polymorphonuclear cell, morphonuclear cell, and erythrocyte Zinc were not found to be reliable enough to act as biomarkers of Zinc status (Lowe et al., 2009). All the above biomarkers have, however, been said to be inadequate to be used for individual

assessment or diagnosis of Zinc deficiency. They can, however, be used for comparison of mean Zinc levels among populations for research purposes. In this vein, this research makes use of serum levels of Zinc in representative samples of case and control groups (Lowe et al., 2009).

There is a variety of methods that can be used to determine serum levels of Zinc including Mass Spectrometry, Inductively Coupled Plasma (ICP), Atomic Absorption Spectrophotometry (AAS), and other classical methods. This study utilizes the method of Atomic Absorption Spectrometry (AAS). Atomic absorption spectroscopy (AAS) can be described as a spectro-analytical procedure for quantitative determination of chemical elements using the absorption of optical radiation (light) by free atoms in the gaseous state. This method was found to be the simplest and least expensive, and yet accurate enough to detect the trace concentrations of Zinc. It is quick and convenient, and it is probably why most of the similar studies reviewed have utilized this method. The equipment that will be used is the Flame Atomic Absorption Spectroscopy which is capable of detecting to the accuracy of parts per billion (or ppb).

As clearly seen from the above discussed studies, there is still controversy and obscurity in the relationship that lies between serum Zinc levels and hypertensive disorders. This presents a research gap and need for more studies in different settings and environments to add to the existing knowledge in the subject of Zinc and preeclampsia. In view of the foregoing, a need was seen for such a study to be conducted in the local Zambian setting to help us compare with the findings in other regions and thus establish external validity of the prevailing conclusions to our own environment and settings.

CHAPTER THREE

METHODOLOGY

3.1 Study design

A comparative cross-sectional study design was found suitable to be used to compare mean serum Zinc levels of preeclamptic and non-preeclamptic pregnant women (Krickeberg et al., 2012).

3.2 Study setting

The study was conducted at the Maternity Clinic of the University Teaching Hospital (UTH), specifically, Ward B02 (antenatal clinic) and Ward B12 (Labor Admission Ward)**.** UTH is the largest hospital in Zambia and is referral to all hospitals in the country.

3.3 Study population

Pregnant women at UTH antenatal clinic and labor ward who met the eligibility criteria for either the control group or study group respectively were included in the study.

3.4 Target population

The target population included Zambian women who were pregnant for more than 20 weeks attending antenatal clinic or labor ward during the period of the study, i.e. February 2016-May 2016 at UTH.

3.5 Inclusion criteria

(For all participants)

- Women pregnant > 20 weeks
- Above 18 years old
- Zambian
- Willingness to participate in study

(For cases only)

- Hypertensive (blood pressure > 140/90 mm Hg)
- Protenuria presence at least 1+

3.6 Exclusion criteria

(For all participants)

- Diabetes
- Taking of mineral supplements
- Non-Zambians
- Minors below 18 years old

(For cases only)

- Presence of hypertension before pregnancy
- Those on antihypertensive therapy

3.7 Sample size calculation

For the determination of the sample size, the formula below, for comparative research studies of comparisons of means, was used as follows:

$$N = \frac{4\sigma^2 \left(Z_{crit} + Z_{pwr}\right)^2}{D^2}$$

N = the sum of the sizes of both comparison groups

σ = Assumed standard deviation = 17.6 percent or 0.176

D = Minimum expected difference between two means = 10 percent or 0.1

Z_{crit} = 1.96 for α = 0.05 or 95 percent confidence level

Z_{pwr} = 0.842 for statistical power 80 percent

Inserting the above figures in the formula gave a sample size, N, equal to 97.23 (or 98) including both comparison groups of preeclamptic and non-preeclamptic pregnant women.

3.8 Sampling method

Participants for this study were purposively sampled.

3.9 Variables

Table 1 Measurement of variables

Type of Variable	Definition of the Variables	Scale of Measure
Dependent Variables • Preeclampsia • Blood pressure	Preeclampsia- (pregnant women with blood pressure that is ≥ 140 mm Hg systolic and/or ≥ 90 mm Hg diastolic and positive proteinuria)	Categorical
	Diastolic and systolic blood pressure measurements	Continuous
Independent variables • Zinc levels	plasma Zinc concentration(measured using AAS in microgram/litre)	Continuous
• Maternal age	Age at last birthday (years)	Categorical
• Gestational age	Number of weeks of the pregnancy	Categorical
• Family history of PE	Presence of close relatives known to have suffered preeclampsia	Categorical
• Residential Area density	High, medium, or low density areas	Ordinal

3.10 Data collection

A midwife recruited for the study measured systolic blood pressure (SBP) and diastolic blood pressure (DBP) of a participant using an automated sphygmomanometer. Two readings were taken and the average recorded for use in the analysis. Demographic data including maternal age, gestational age, residential area, family history of preeclampsia were also recorded in a data collection sheet. Urine samples were collected for the purpose of diagnosing proteinuria in urine bottles. Urine dipstick proteinuria of 1+ or more was taken as positive proteinuria. Participants with systolic blood pressure above 140 mm Hg and/or diastolic blood pressure above 90 mm Hg with positive proteinuria were considered to be the preeclamptic cases. Control participants (non-preeclamptic) comprised pregnant women without hypertension or proteinuria but attending regular Antenatal clinic (ward B02).

To prepare serum, 8 mls of blood was drawn from the cubital vein using a sterile needle and syringe into an appropriate tube. The blood samples were then centrifuged (Allegra 6R, Beckmann Coulter, USA) in the plain tubes for 10 min at 4000 rpm and the resulting serum was drawn and stored at -20°C until the stage of Zinc analysis.

The serum collected from both groups for all participants was assayed for concentration of Zinc in microgram per deciliter using AAS technique at the Food and Drugs control laboratory. Data was recorded in a data collection sheet in readiness for analysis.

3.11 Method of serum Zinc determination

Calibration

Calibration standards were prepared from Multi – Element Calibration standards traceable to NIST in intermediate ranges of:

0, 25, 50, 100, 150, 200, 250 µg/L from 100 ppm stock standard

Calibration Graph:

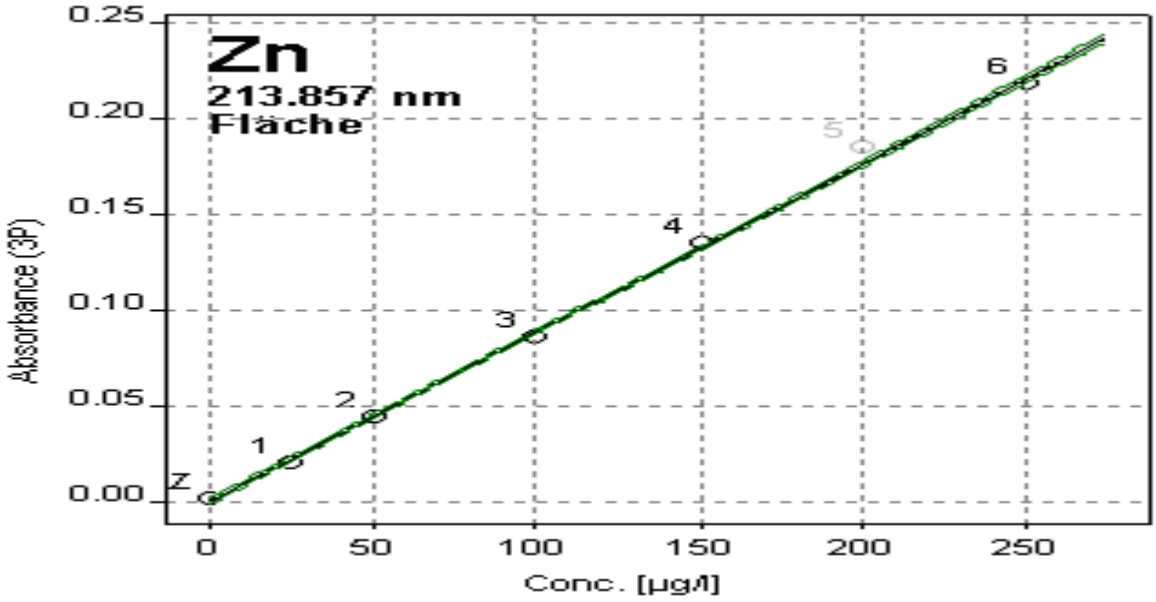

Figure 1 Zinc Absorbance curve

Acceptable Correlation Coefficient: $R^2 = 0.985$ µg/L

Procedure

- For the determination of Zn, the blood samples were centrifuged at 400 rpm for 3 minutes to separate serum.
- 0.5 ml of serum was diluted to 10 ml with deionised water
- Diluted samples were treated with 1 ml of 0.1 percent KCl

The dilution factor was 20.

After the Reagents were added, the samples were ready to be analysed using flame AAS.

The measurements were performed with the Contr AA 700

Table 2 Flame characteristics

Element	Wavelength	Flame Type	Gas Flow Rate L/h	Burner Height (mm)
Zn	213.8570	Air /C_2 H_2	45	8

The obtained results were recorded in Microsoft excel.

3.12 Data analysis

Data was analyzed in parts using stata version 14 statistical package. The first part involved coming up with summary statistics for both comparison groups in terms of the demographic variables that were collected by way of a questionnaire. Also, a comparison of the mean serum Zinc levels in both comparison groups in general. The second part involved matched analysis of serum Zinc level in both comparison groups with respect to other variables that are believed to be confounding. In all comparisons, significance was determined at $p = 0.05$. Lastly, a Pearson correlation was run between SBP and serum Zinc level and also DBP and serum Zinc level in both comparison groups and correlation coefficients were used to interpret the relationships.

3.13 Study limitations

We were faced with a few limitations worth mentioning. Due to lack of adequate funding, the study did not assay for other minerals and metabolites that are also believed to influence the occurrence of preeclampsia. Additionally, the influence of blood albumin levels and of dietary fluctuations on the mineral status of the participants was not measured.

3.14 Ethical considerations

The information gathered from participants was treated with utmost confidentiality. Moreover, assurance was given to the participants that the results of the study would be disseminated to relevant recipients with no direct link to them and anonymity would be strictly adhered to. However, the ultimate benefits on health and science that the study would bring were also communicated to the participants.

Only willing participants were recruited. Adequate information about the study was given using an information sheet (Annex 1.3) and a consent form (Annex 1.4) which they were required to sign in the presence of a witness.

The slight risk of pain and discomfort that would occur during blood collection was also explained beforehand, but participant were assured that only qualified medical personnel would handle the collection of blood and that they would be taken care of in the unlikely event of an emergency.

The protocol was approved by ERES CONVERGE IRB and UTH management before the practical research commenced.

CHAPTER FOUR

RESULTS

4.1 General demographic characteristics of the preeclamptic and non-preeclamptic participants

Refer to Table 3 below.

Table 3 Demographic statistics for the preeclamptic pregnant women and the non-preeclamptic control participants

Variables	Preeclamptic participants *n=41* (mean ± SD)	Non-preeclamptic participants *n=57* (mean ± SD)	P-value
Age group (years)			
18-30	32 ± 47%	60 ± 50%	0.006
30-40	61 ± 49%	37 ± 49%	0.018
40+	7.3 ± 26%	4 ± 19%	0.403
Gestational Age (weeks)			
20-25	12 ± 33%	26 ± 44%	0.088
25-30	24 ± 43%	4 ± 19%	0.002
30+	63 ± 49%	70 ± 46%	0.487
Family History of preeclampsia			
No	85 ± 36%	82 ± 38%	0.704
Yes	15 ± 36%	18 ± 38%	0.704
Residential Area Density			
High	51 ± 51%	56 ± 50%	0.634
Low	12 ± 33%	16 ± 37%	0.620
Medium	37 ± 49%	28 ± 45%	0.377
Severity of Preeclampsia (%)			
Mild	26.83		
Moderate	19.51		
Severe	53.66		

Source: This study

4.2 Comparison of serum Zinc levels in the preeclamptic verses non-preeclamptic women

Table 4 Serum Zinc levels in the total sample of preeclamptic verses non-preeclamptic women

	Preeclamptic group (n = 41)	Non-preeclamptic group (n = 57)
Serum Zinc conc. (µg/dL)	89.17 ± 47.19	76.20 ± 35.23

4.3 Comparisons of serum Zinc levels of preeclamptic verses non-preeclamptic women within matched demographic categories

The results for the matched comparisons of serum Zinc levels in the preeclamptic verses non-preeclamptic based on matched variables are summarized in Table 5 below.

Table 5 Comparison of mean serum Zinc levels in preeclamptic verses non-preeclamptic pregnant women in various categories

Variables		Serum Zinc Concentration levels (µg/dL)		**P-value**
		n=41	*n=57*	
		Pre-eclamptic (mean ± SD)	Non-Pre-eclamptic (mean ± SD)	
Age group (years)	18-30	87.11 ± 35.29	76.84 ± 37.09	0.3941
	30-40	94.32 ± 52.77	76.69 ± 34.10	0.1948
	40+	55.19 ± 39.59	60.06 ± 22.03	0.8876
Gestational Age (Weeks)	20-25	76.56 ± 22.66	74.48 ± 22.31	0.8590
	25-30	86.61 ± 25.51	67.16 ± 11.99	0.3296
	30+	92.59 ± 56.58	77.29 ± 39.97	0.2025
Family History of pre-eclampsia	Yes	81.95 ± 38.94	71.65 ± 18.03	0.4784
	No	90.41 ± 48.85	77.16 ± 38.02	0.1710
Residential Area Density	High	92.791 ± 58.62	83.27 ± 43.42	0.500
	Medium	87.83 ± 32.52	67.26 ± 19.18	*0.0391*
	Low	78.02 ± 38.06	66.90 ± 16.12	0.4161

4.4. Correlation of Zinc level and blood pressure in preeclamptic and non-preeclamptic participants

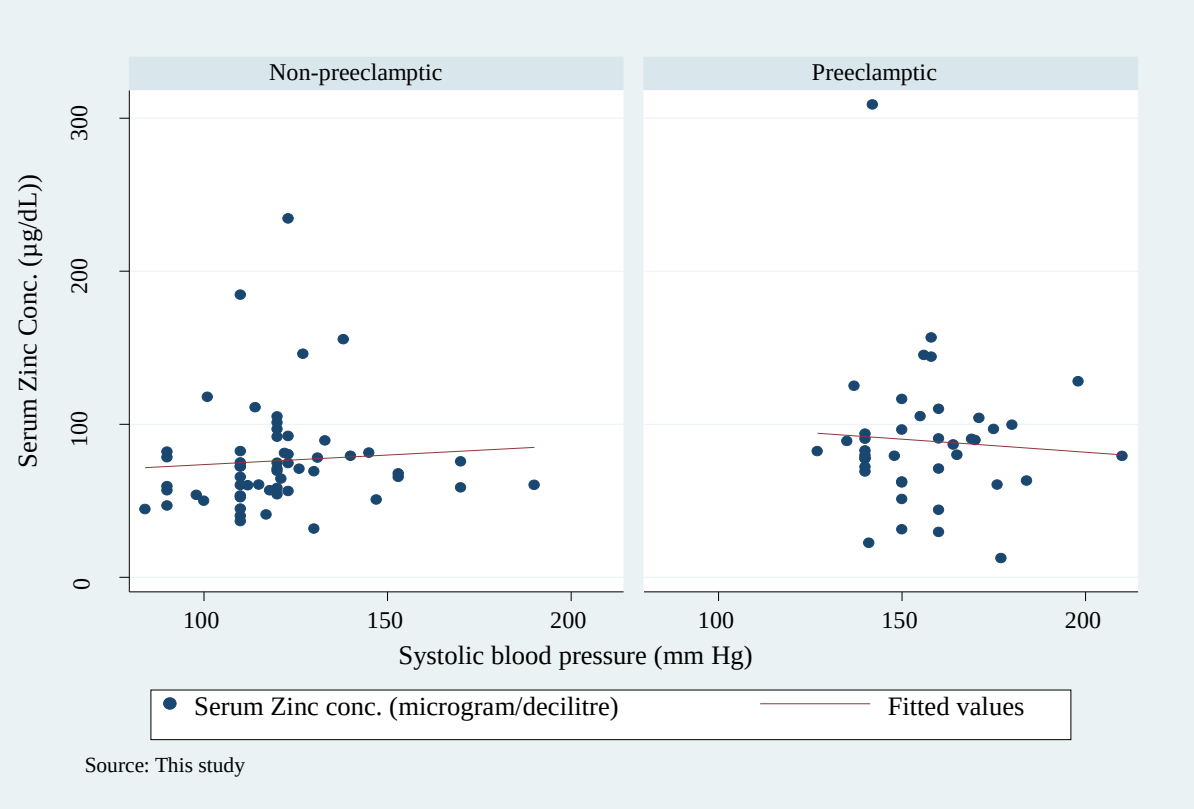

Figure 2 Correlation of SBP and serum Zinc level in both comparison groups

Figure 2 shows that the non-preeclamptic had a positive correlation (r = 0.1149; p = 0.2599) while the preeclamptic had a negative correlation (r = -0.0636; p = 0.6928) between serum Zinc level and SBP. However, the correlation coefficients were not significant at 95 percent confidence level. Refer to Table 6.

Figure 3 Correlation of DBP and serum Zinc level in both comparison groups

Figure 3 shows that both comparison groups had a positive correlation with serum Zinc level, but the coefficients of correlation are equally insignificant (r = 0.1656; p = 0.1031 for non-PE, and r = 0.0452; p = 0.7788 for PE group). Also refer to Table 6 below.

Table 6 Correlation coefficients of DBP and SBP verses serum Zinc level in the non-preeclamptic controls and in preeclamptic cases (*a* and *b*).

(a) Non-preeclamptic controls

	Serum Zinc Conc. (μg/dL)		
Variable	n	correlation coefficient	p-value
SBP	57	0.1149	0.2599
DBP	57	0.1656	0.1031

(b) Preeclamptic cases

	Serum Zinc Conc. (μg/dL)		
Variable	n	correlation coefficient	p-value
SBP	41	-0.0636	0.6928
DBP	41	0.0452	0.7788

CHAPTER FIVE

DISCUSSION

It can be deduced from the above illustrated data that the findings show no significant association between serum Zinc level and blood pressure in preeclamptics or non-preeclamptics. It might therefore follow that changes in serum Zinc levels do not play a direct role in the pathogenesis of preeclampsia. In our study serum Zinc levels were found to have no significant effect on systolic or diastolic blood pressure. There was a positive correlation between the serum Zinc levels and blood pressure though insignificant.

Our results are supported by the findings of some other studies. In one study where 20 preeclamptic and 20 healthy women were recruited it was reported that serum Zinc level was slightly lower in the preeclamptic than the healthy pregnancies, but the difference was not statistically significant (Adam et al., 2001b). It was argued that the lower serum Zinc levels reported in other studies could be due to other factors, other than Zinc deficiency, such as reduced albumin concentrations. Magri et al., (2003) did not find a relationship between the serum levels of Calcium, Magnesium, and Zinc and gestational hypertension; therefore, they proposed that these elements might not clinically participate in the pathogenesis of the gestational hypertension (Magri et al., 2003). Their findings and conclusions are compatible with the results of the present study as far as serum Zinc is concerned. However, in our study a higher mean Zinc concentration was unexpectedly found in the preeclamptic rather than the normal controls.

Others have reported significantly lower mean serum Zinc in preeclamptic patients than in normal pregnancies and an inverse correlation of blood pressure and the mineral levels in pregnant (Kim et al., 2012, Kumru et al., 2003). The studies done in Nigeria and Iran also

concluded that levels of Zinc were significantly low in preeclampsia and found a significant difference in the levels in cases verses controls ($p < 0.0013$), (Akinloye et al., 2013).

This lack of consensus on the role of Zinc on pregnancy outcome is largely due to contradictory results from researchers. Although many results seem to suggest that deficiency of serum Zinc may predispose to preeclampia (Akinloye et al., 2013) other results have demonstrated no association at all (Amirabi et al., 2015). This can be due methods for measuring serum Zinc not being sensitive or specific enough, variations in sample sizes and many other factors including genetic differences (King, 2000).

Generally one would expect low serum Zinc levels to be associated with preeclampsia as zinc has got significant antioxidant properties, which minimizes oxidative stress, one of the main factors associated with preeclampsia (Hubel, 1997). The role of Zinc binding proteins may also play a major role in total serum Zinc in pregnancy. For instance some researchers have demonstrated that hypoalbuminemia can predispose to reduced serum Zinc levels and it has also been observed that in pregnancy the affinity of albumin for Zinc tends to be reduced which can also predispose to low serum Zinc level (Giroux et al., 1976). What this means is that serum Zinc levels may be low in the presence of normal tissue levels because of reduced transport proteins.

The controversy continues as to whether trace minerals such as Zinc are actually significantly involved in pathogenesis of preeclampsia, but it is quite obvious that geographical and nutritional differences play a role in the different Zinc levels that have been reported by various studies. While the results of various studies are controversial, it has also been argued that serum Zinc may be under tight homeostatic regulatory mechanisms that keep the level fairly constant in spite of nutritional or pathological changes (Lowe et al., 2009). Zinc has been thought to be an important intermediary factor in preeclampsia as it is required for the

proper functioning of antioxidant enzymes which protect the epithelial lining of blood vessels from free radicals injury. Imbalance of oxidants and antioxidants in favour of the former in maternal vascular systems has been established as a hallmark of preeclampsia (Palei et al., 2013).

According to one study, nutritional supplementation of Zinc was associated with lower incidence of preeclampsia though plasma Zinc levels remained fairly constant (Adam et al., 2001b). It could therefore be possible that Zinc plays a role in preeclampsia but serum Zinc level may not be the appropriate way of detecting that role (Adam et al., 2001b).

It is worth noting that in our study maternal age was found to be a related risk factor of preeclampsia. Mothers between 30 and 40 years of age were at a higher risk of developing preeclampsia compared with mothers between 18 and 30 years old. Underlying mechanisms for this are not fully understood and further research is called for. This phenomenon was also observed in previous studies (Jolly et al., 2000) and is probably due to the fact that older mothers have aging blood vessels and therefore prone to uterine hypertension.

Some researchers have reported that preeclampsia is more common in poor women and those with family history of the disease. Explanations have been proposed that poor women usually have a low intake of foods rich in essential minerals but high in phytic acid (i.e. unprocessed cereals and legumes) and therefore are more vulnerable to deficiencies which may be linked with preeclampsia (Gibson, 2012). It has also been proposed that preeclampsia could be associated with genotype, hence runs in families. Though a positive family history has been demonstrated in patients in some studies highlighting a complex genetic background (Carr et al., 2005, Smith et al., 2009) our study didn't reveal a positive association. This could have been due to ignorance about medical histories of relatives by our study participants. Most preeclamptic were above 30 years and this is in line with what has been observed that in most

cases preeclampsia starts manifesting itself in the second half of pregnancy specifically after 20 weeks of pregnancy (Feulner, 2015). Our study did not show differences between women from low or high residential areas or between women with or without family history of preeclampsia in terms of Zinc level or prevalence of preeclampsia. The above observation is in line with a review study by Sibai et al (1998) which expressed despair at the endeavours to find a link between Zinc levels and preeclampsia or efforts to use its supplementation for prevention purposes (Sibai, 1998). However research for associated risk factors of this disease continues as it may help in the determination of early predictors of preeclampsia.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

The study has established that the mean serum Zinc concentration in the preeclamptic study group was 89.17 ± 47.19 µg/dL while in the non-preeclamptic control group it was 76.20 ± 35.23 µg/dL. However, the two mean serum Zinc levels were not statistically different ($p = 0.122$). Additionally, maternal age, gestational age, history of preeclampsia, and residential area density did not show any significant effect on the difference in the serum Zinc levels of the preeclamptic verses non-preeclamptic pregnant women. It can therefore been concluded that serum Zinc level has no clinical value in preeclampsia.

Additionally, it can be concluded based on the results of Pearson's correlation that there was no linear relationship of any significance between serum Zinc level and blood pressure in the total sample of participants or in preeclamptic women alone.

However, the present study has demonstrated that pregnant women of 30 – 40 years of age are at a higher risk of experiencing preeclampsia than women of 18 – 30 years of age. This means that in order to evade the risk of preeclampsia for women above 30 years, earlier biomarkers of a preeclamptic state and better management of those with the pre-condition need to be developed.

Further research is recommended to determine the effect of Zinc supplementation and/or effective dietary modifications on the outcome of preeclampsia in the Zambian population. Additionally, future studies should consider isotope studies as a more accurate way to detect mineral levels.

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1.0. ANNEX

1.1 QUESTIONNAIRE (INTERVIEW GUIDE)

Date:……..Participant

ID:……

1. Age

   (A) 18-30 (B) 30-40 (C) Above 40

2. Age of pregnancy

   (A) 20-25 wks (B) 25-30 wks (C) Above 30 wks

3. Area of residence

   (A) High density (B) Medium density (C) Low density

4. Did you have hypertension before pregnancy?

   (A) Yes (B) No

5. History of Pre-eclampsia in the family?

   (A) Yes (B) No

6. Do you take any antihypertensive drugs?

   (A) Yes (B) No

1.2 DATA SHEET

Participant ID	Weight (Kg)	Height (m)	BMI Kg/m^2	Proteinuria	Blood Pressure (mm Hg)	Glucosuria	Serum Zinc level (ppb)

1.3 Information sheet

Title of Study; Relationship between Serum Zinc levels and Preeclampsia in Zambian pregnant women

Dear Participant,

This is to inform you that this study is being carried out by Chababa Likando, a postgraduate student at the University of Zambia, School of Medicine; Department of Physiological Sciences. The health of pregnant women should be a concern for every one of us as they are going through a special period of time of bringing a new life into the world. Studies in other countries have indicated that disturbances of Zinc levels may be a mediatory factor in the development of Preeclampsia (Onset of hypertension and proteinuria after 20 weeks of pregnancy). Preeclampsia is risky for the health and life of both mother and child. Research efforts are therefore needed to come up with information that can lead to alleviation of this problem.

You are invited to be involved in this study for a maximum period of only 2 days (Day 1 – enrollment/ questionnaire and Day 2 – specimen sample collection). In this study, 8 mls of blood will be collected then subsequently subjected to centrifugation to separate serum which will then be stored at -20 degrees Celsius awaiting laboratory analysis of Zinc level. In the same vein, the 10 ml urine samples will be used for diagnostic purposes of Preeclampsia. All specimens will be safely discarded after use.

There are physical risks of pain and discomfort when collecting blood; however you should not worry because you will be attended to by qualified doctors and nurses. The blood examination will be used to gather information which will contribute to management of hypertension that occurs in pregnancy. Your participation in this study is purely voluntary. You are free not to answer questions deemed personal or sensitive. Additionally, you are

eligible to withdraw if you are not interested and your action will not affect your acquisition of health services. Please seek clarification where you do not understand.

All the information you will provide will be strictly confidential. The research information will be disseminated to the relevant authorities and with no direct link to you since anonymity shall be maintained.

In the event of any problems, please contact the following:

1. Chababa Likando, University of Zambia, School of Medicine, Department of Physiological Sciences, P.O. Box 50110, Lusaka, Zambia. Mobile Phone; 0973374313

2. The Secretary, ERES Converge, 33 Joseph Mwilwa Road, Rhodes Park, Lusaka, Tel: +260 955 155 633, +260 955 155 634, +260 966 765 503, Email: eresconverge@yahoo.com.

1.4 Consent form

CONSENT TO PARTICIPATE IN THE STUDY

Study Title: Relationship between Serum Zinc levels and Preeclampsia in Zambian pregnant women.

By signing my name below, I ..... confirm the following:

I have read (or had read to me) this entire consent document and all of my questions have been answered to my satisfaction.

The study's purpose, procedures, risks and possible benefits have been explained to me.

I agree to let the study team use and share the health information and other information gathered for this study. I voluntarily agree to participate in this research study and I agree to give small amount (8mls) of blood.

Participant signature…… **Date**……

Thump Print below:

IMPORTANT: You will receive a signed and dated copy of this consent form. Please keep it where you can easily find it. It will help you remember what we discussed today.

Statement by the researcher/ person taking consent:

I have accurately read out the information sheet to the potential participant, and to the best of my ability made sure that the participant understands the research procedure and they are free to withdraw from the study at any time without penalty.

I confirm that the participant was given an opportunity to ask questions about the study, and all the questions asked by the participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

Name of Person Taking Consent: _____ Signature: _____

Name of witness: _____ Signature: _____

Date _____ Day/month/year

PERSONS TO CONTACT in case of problems or clarification

1. Chababa Likando, B.Sc, University of Zambia, School of Medicine, Department of Physiological Sciences, P.O. Box 50110, Lusaka, Zambia. Mobile Phone; 0973374313.

2. The Secretary, Eres Converge IRB, 33 Joseph Mwila road, Roads park, Lusaka. Email: eresconverge@yahoo.co.uk Phone: 0955155633/4