EVALUATION OF COPPER, ZINC AND SELENIUM IONS IN PREGNANT WOMEN WITH OR WITHOUT PREECLAMPSIA IN A TERTIARY HEALTH CLINIC IN BENIN CITY, EDO STATE.

BY

OYANA MICHAEL CHUDE

BMS2001203

UNIVERSITY OF BENIN

BENIN CITY

SEPTEMBER, 2025

EVALUATION OF COPPER, ZINC AND SELENIUM IONS IN PREGNANT WOMEN WITH OR WITHOUT PREECLAMPSIA IN A TERTIARY HEALTH CLINIC IN BENIN CITY, EDO STATE

BY

OYANA MICHAEL CHUDE

BMS2001203

DEPARTMENT OF MEDICAL LABORATORY SCIENCE,

SCHOOL OF BASIC MEDICAL SCIENCES,

UNIVERSITY OF BENIN,

BENIN CITY

SEPTEMBER, 2025

EVALUATION OF COPPER, ZINC AND SELENIUM IONS IN PREGNANT WOMEN WITH OR WITHOUT PREECLAMPSIA IN A TERTIARY HEALTH CLINIC IN BENIN CITY, EDO STATE

BY

OYANA MICHAEL CHUDE

BMS2001203

BEING A PROJECT SUBMITTED TO THE DEPARTMENT OF MEDICAL LABORATORY SCIENCE IN PARTIAL FULFILLMENT FOR THE REQUIREMENT OF THE AWARD OF BACHELOR'S DEGREE IN MEDICAL LABORATORY SCIENCE(B.MLS) UNIVERSITY OF BENIN, BENIN CITY, NIGERIA.

SUPERVISED BY:

PROF.M.A. EMOKPAE

SEPTEMBER,2025

CERTIFICATION

This is to certify that this research project was carried out by **OYANA MICHAEL CHUDE** with matriculation number **BMS2001203** under the supervision of **Prof. M.A. EMOKPAE** and was submitted to the Department of Medical Laboratory Science, School of Basic Medical Sciences, University of Benin, in partial fulfillment of the requirement for the award of Bachelor of Medical Laboratory Science (B.MLS) Degree.

…….

Prof. M.A. EMOKPAE

Supervisor

…….

DATE

……

Dr. Mrs. ZAINAB OMORUYI

Head of Department

.....

DATE

……

EXTERNAL EXAMINER

……

DATE

DEDICATION

This research project is dedicated to God Almighty for making this project a success and also to my lovely Mum, Mrs. N.E. Oyana for her motherly advice and support to make this milestone in my academic journey a reality.

ACKNOWLEDGEMENT

My deepest gratitude goes to God Almighty for the wisdom, understanding He bestowed upon me and also for his provision during the course of this research study.

My sincere appreciation goes to my supervisor Prof. M.A. EMOKPAE for his expertise and guidance in this research study.

Special thanks to the Head of Department, Medical Laboratory Science, Dr. Mrs. Zainab Omoruyi for providing ample time for the completion of this study.

My deep sense of gratitude goes to my parents for their love, support, encouragement and prayers.

I also express my gratitude to Dr. Bright Odozi and the Antenatal Clinic Laboratory of University of Benin Teaching Hospital (UBTH) and also Dr. Timothy of the Department of Science and Laboratory Technology for their aid in the successful achievement of this research study.

To my group partner Goodluck and everyone who aided my success in this research work, I say a very big thank you all for the support. God bless you all.

TABLE OF CONTENTS

COVER PAGE i

FRONT PAGE ii

TITLE PAGE iii

CERTIFICATION iv

DEDICATION v

ACKNOWLEDGEMENT vi

TABLE OF CONTENTS vii

LIST OF TABLES xi

LIST OF FIGURES xii

ABSTRACT xiii

CHAPTER ONE 1

INTRODUCTION 1

1.1 Background of Study 1

1.2 Justification of the study 6

1.3 Aim of the Study 8

1.4 Specific Objectives of the Study 8

1.5 Scope of Study 9

1.6 Research Hypotheses 9

1.7 Research Questions 11

CHAPTER TWO 12

LITERATURE REVIEW 12

2.1 Preeclampsia 12

2.1.1 Pathogenesis 13

2.1.2 Clinical Signs and Symptoms 14

2.1.3 Diagnosis of Preeclampsia 15

2.1.4 Treatment / Management of Preeclampsia 17

2.2 Antioxidants 18

2.3 Essential Trace metals / Essential Trace elements 19

2.3.1 Copper (Cu) 22

2.3.1.1 Dietary Sources of Copper 23

2.3.1.2 Absorption, Transport, Metabolism and Excretion of Copper 23

2.3.1.3 Deficiency of Copper 25

2.3.1.4 Toxicity of Copper 25

2.3.1.5 Relationship between Preeclampsia and Copper Homeostasis 26

2.3.2 Zinc (Zn) 27

2.3.2.1 Dietary Sources of Zinc 28

2.3.2.2 Absorption, Transport, Metabolism and Excretion of Zinc 28

2.3.2.3 Zinc Deficiency 29

2.3.2.4 Zinc Toxicity 30

2.3.3 Selenium (Se) 32

2.3.3.1 Dietary Sources of Selenium 33

2.3.3.2 Absorption, Transport, Metabolism and Excretion of Selenium 33

2.3.3.3 Selenium Deficiency 33

2.3.3.4 Selenium Toxicity 34

2.3.3.5 Relationship between Preeclampsia and Selenium Homeostasis 34

CHAPTER THREE 36

MATERIALS AND METHODS 36

3.1 Study Area 36

3.2 Study Design 36

3.3 Study Population 36

3.4 Sample Size 37

3.5 Laboratory Analysis 38

3.5.1 Principle of Atomic Absorption Spectrometry (AAS) 38

3.6 Sample Preparation 40

3.7 Procedure 41

3.8 Introduction of the Sample into the analyzer of Atomic Absorption Spectrophotometer 41

3.9 Quality Control 43

3.10 Statistical and Data Analysis 43

CHAPTER FOUR 44

RESULTS 44

4.0 Results 44

CHAPTER FIVE 53

DISCUSSION 53

5.1 Discussion of Findings 53

5.2 Conclusion 55

5.3 Recommendations 56

REFERENCES 57

LIST OF TABLES

Table 4.1: Shows the serum concentrations/ levels (µg/dl) of Cu, Zn and Se in pregnant preeclamptic and non-preeclamptic groups. 44

Table 4.2: shows the mean concentrations of selenium (Se), copper (Cu) and zinc (Zn) in pregnant women with or without preeclampsia. 49

Table 4.3: shows the estimated means of selenium, copper and zinc levels in the control and preeclamptic groups. 51

LIST OF FIGURES

Figure 3.1: Diagrammatic representation of the working principle of the Atomic Absorption Spectrometry (AAS) 40

Figure 3.2: Graphical illustration of the mean levels of serum selenium, copper and zinc among pregnant women without preeclampsia 52

ABSTRACT

Preeclampsia is a pregnancy-specific hypertensive disorder associated with significant maternal and fetal morbidity and mortality. Increasing evidence suggests that changes in trace elements, particularly copper (Cu), zinc (Zn), selenium (Se), may contribute to endothelial dysfunction and oxidative stress in preeclampsia pathogenesis. The aim of this study was to evaluate serum concentrations of Cu, Zn, and Se in pregnant women with and without preeclampsia attending a tertiary health clinic in Benin City, Edo State, Nigeria. A case –control design was employed involving 80 participants consisting of clinically diagnosed preeclamptic patients and age- and gestational –age –matched normotensive pregnant women. Venous blood samples were collected, and serum Cu, Zn and Se levels were determined using Atomic Absorption Spectrometry (AAS). Data were analyzed using appropriate statistical tools such as the student t test, with significance set at $p<0.05$. Preliminary findings indicate that preeclamptic women exhibit significantly higher Cu levels and reduced Zn and Se concentrations compared to controls, suggesting an imbalance between pro-oxidant and antioxidant trace elements. These alterations may exacerbate oxidative stress, contributing to disease severity. The results underscore the importance of Cu, Zn, and Se as potential biochemical markers in the assessment and management of preeclampsia. Further large –scale studies are recommended to explore their predictive and therapeutic implications in pregnancy –related hypertensive disorders.

Keywords: Copper, Zinc, Selenium, Preeclampsia, Pregnancy, Oxidative Stress.

CHAPTER ONE

INTRODUCTION

1.1 Background of Study

Preeclampsia has a long history of being a major cause of maternal and perinatal morbidity and mortality worldwide especially in developing regions such as Nigeria (Tanner et al., 2022). In Nigeria, preeclampsia is a hypertensive disorder that affects pregnant women and is mainly characterized by proteinuria and high blood pressure after the 20th week of gestation (Oyeyinka & Olaniyan, 2024). The so-called classic triad of hypertension, proteinuria and edema have been superseded with hypertension and organ dysfunction, be it renal, hepatic, hematological, neurologic, or placental, being sufficient for diagnosis (Tanner et al., 2022).

Preeclampsia as a hypertensive disorder of pregnancy occurs after 20 weeks of gestation. It has oxidative stress as its major implication. The features of preeclampsia include elevated blood pressure greater than 140/90 mmHg, proteinuria greater than 300mg per 24 hours urine collection ,albumin-creatinine ratio of more than 0.3 mg/dl, urinalysis dipstick reading of 2+, renal insufficiency with serum creatinine concentration greater than 1.1 mg /dl, impaired liver function of elevated plasma concentration of liver transaminases, lactate dehydrogenase(LDH), oxidative stress induced haemolysis, decreased platelet count (Narkhede & Karnad, 2021).

In Oxidative stress, there is increased physiological demand for antioxidants such as Vitamins A, C and E, Trace elements and other bioactive compounds such as polyphenols, flavonoids, glutathione especially during physiological conditions such as pregnancy. Essential trace elements are micronutrients which are catalytic substances needed by the human body in small amount in form of metallic ions (Otebhi & Osadolor, 2019). Trace elements like selenium (Se),

copper (Cu), zinc (Zn) are cofactors of antioxidant enzymes like, superoxide dimutases, glutathione peroxidases (GPX), thioredoxin reductases (TRXR) (Goularte et al., 2025).

Copper and Zinc ions in the maternal blood plasma are known for their antioxidant and anti-inflammatory roles (Grzeszczak et al., 2020).They are known to influence enzymatic activities, regulate gene expression and synthesize proteins in pregnancy (Grzeszczak et al., 2020). In pregnancy, copper and zinc ions are needed for fetal growth and development. Thus, they reduce the risk of complications such as hypochromic anemia, pregnancy induced hypertension, low birth weight, postnatal complications (Grzesczak et al., 2020). Copper ions is important in pregnancy for physiological and biochemical processes such as erythropoiesis, collagen, melanin, and myelin synthesis, regulation of redox reactions, iron metabolism and transport, fatty acids metabolism (Grieger et al.,2019). However, the toxicity and deficiency of copper ions in the maternal blood plasma or altered copper homeostasis is associated with pregnancy induced complications. For instance, hypercupremia (increased plasma concentration of copper ions greater than 140 µg /dl) may lead to wilson disease, fenton reaction(formation of reactive oxidative species, hydroxyl radicals that damages fetal DNA, proteins and lipids through the promotion of oxidative stress and peroxidation), pre-term births, preeclampsia, low birth weight, postnatal complications such as gestational diabetes while hypocupremia (decreased plasma concentration of copper ions less than 70 µg /dl) may lead to hypochromic anemia, intrauterine growth restriction, spontaneous abortion (Grzesczak et al.,2020).

Zinc ions in maternal and placental circulation during pregnancy is essential for cell division and differentiation for fetal cells and organs development, fetal bones mineralization, regulation of fetal and maternal homeostasis, ,regulation of the secretion of hormones such as growth hormones, insulin, testosterone, thymulin a thymus hormone needed for immune functions,

activation of metalloproteases and antioxidant enzymes such as superoxide dismutase, glutathione peroxidase that eliminates and scavenges free radicals, inhibition of unsaturated fatty acids, antagonisation of heavy metals like cadmium ,lead, detoxification of reactive oxidative species, nitrogenous compounds, organic compounds, embryogenesis and fetus development (Grzeszczak et al,2020). Zinc in the form of zinc oxide supplements reduces the generation of inflammatory cytokines and chemokines like C-reactive protein (CRP), interleukins such as IL -6, monocyte chemo-attractant protein (MCP-1), secretory cell adhesion molecules. Thus zinc reduces the incidence of maternal infections, levels of oxidative stress and inflammatory markers such as CRP, adrenocorticosteroids in pregnancy. Zinc is a regulator of NF-kb pathway in maternal and placental immunity thus zinc plays a critical role in maternal and placental immunity, inflammation during pregnancy by regulating the activation of cytokines, chemokines and adhesion molecules such as IL-1β,IL-6,IL-8,TNF-α,CRP, fibrinogen ,selectins and integrins . Therefore, zinc alongside selenium are needed to sustain immuno-competence in pregnancy (Kumari et al., 2022). However, the toxicity and deficiency of zinc increases the risk of pregnancy induced complications such as preeclampsia. Studies show that decreased plasma concentration of zinc less than70 µg/dl can lead to hypercupremia due to a greater competitive binding between copper and zinc ions to transport molecules in the placental and maternal circulation thus leading to preeclampsia (Grzeszczak et al., 2020). According to Kumari et al. (2022), pregnant women are considered a high risk group for zinc deficiency due to decreased intestinal absorption of zinc during pregnancy, lifestyle factors such as smoking, alcohol abuse, strenuous exercise, medications. apart from preeclampsia, zinc deficiency in pregnancy can induced prolonged labour, infertility, teratogenesis, impaired placental growth, intrauterine growth restriction, embryonic or fetal death as well as increased anxiety, depression, irritability,

stress, impaired immune functions leading to acute phase reactions induced by inflammation and maternal infections. The deficiency of zinc in pregnancy reduces the activity of thymulin, a hormone responsible for the regulation of placental immunity, induces lymphopenia, inflammation associated vascular dysfunctions. According to Iqbal et al. (2020), there is a linear association between pregnancy complications and zinc deficiency in maternal circulation.

Another trace element of interest in reproductive health is Selenium. It is an essential trace element that is important for ovarian follicular growth and maturation, parturition, implantation (Grieger et al., 2019). Selenium in pregnancy is needed for the regulation of thyroid hormone metabolism, calcium metabolism and synthesis of DNA (Kumari et al., 2022). Also in pregnancy, selenium affects and influences uterine function, embryonic development, conceptous growth, gene and angiogenic factors expression, placental and fetal growth and development, improvement of fetal substrate supply, regulation of production of pro-inflammatory proteins during labour and conception. Selenium in pregnancy is an important antioxidant since it is a component of antioxidant enzymes like selenoproteins (S, P, W), iodothyronine deiodinases, glutathione peroxidases (GPX), thioredoxin reductases (TRXR) which reduces the generation of reactive oxidative species thus prevents fetal DNA damage (Dahlen et al., 2022). The effects of the selenium toxicity in pregnancy are rarely known but according to Duntas (2020) examined, selenium deficiency in pregnancy has been associated with adverse pregnancy outcomes like miscarriages, neural tube defects, pre-term births, low birth weight, retarded fetus intrauterine growth, preeclampsia, gestational diabetes, hernia.

Here, selenium deficiency in the 2nd and 3rd trimester of pregnancy increases the generation of reactive oxidative species due to reduced GPX activity, hypercupremia, causing the hypertensive disorder of preeclampsia that accounts for 15% of maternal deaths and preterm births. Selenium

toxicity and deficiency is associated with infertility which is the inability to conceive and establish pregnancy, poor embryonic development, altered endometrium and uterine function (Dahlen et al., 2022).

In this study, pregnant women represent unique demographic group since they are at high-risk group of developing preeclampsia and other pregnancy related complications as a result of factors such as increased physiological depletions of antioxidant micronutrients during the trimesters of pregnancy, parity state (multiple pregnancy). Other factors such as smoking, chronic alcoholic consumption, exposure to industrial chemicals, industrial fumes, pesticides, heavy metals, medications, insufficient dietary intake, social and economic factors like income, inability to access proper healthcare especially in low- and middle-income earning regions such as Nigeria only worsens this predisposition. In Nigeria, the tendency of a pregnant woman to develop preeclampsia is high due to nutritional deficiencies of micronutrients like trace minerals in diets such as copper, zinc and selenium, increased utilization of micronutrients, low income to access food sources and medications rich in these micronutrients like nuts, seafood, poultry, organ meats, supplements, healthcare facilities for the early detection and diagnosis of preeclampsia (Prasad et al., 2025).

This study therefore intends to explain the roles of copper, zinc and selenium in pregnancy, the changes in their level in maternal circulation as a risk factor of preeclampsia, their potential use as clinical biomarkers in the early diagnosis of preeclampsia and other pregnancy related complications, provide critical data that may influence health interventions aimed at preventing these pregnancy related complications.

1.2 Justification of the study

The increasing prevalence of preeclampsia among pregnant women has become a significant reproductive health concern in Nigeria where the risk factors of this phenomenon are abundant. Such factors include low income to access proper antenatal care, diets rich in micronutrients that can prevent preeclampsia and other related complications, lack of routine diagnostic tools to assess maternal health among others.

Trace elements such as copper, zinc and selenium in the right amount through their homeostasis in maternal circulation have been associated with prevention of preeclampsia though over dosage of these trace elements are also implicated as causative factor of preeclampsia. These trace elements are crucial for maintaining both the maternal and metabolic health of pregnant women due to their antioxidant properties and changes in their homeostasis can be an early indicator of preeclampsia and other related complications. But how their levels in body fluids such as plasma, serum and urine are estimated in order to assess maternal health poses a very challenging limitation in the early diagnosis of preeclampsia, early provision of health interventions.

In Nigeria, studies exploring the relationships between these trace elements and reproductive health are limited especially in the tertiary health institutions where there is a lack of diagnostic assays to assess the levels of copper, zinc and selenium ions for the diagnosis of preeclampsia in pregnancy, lack of governmental assistance in the provision of such assays, lack of governmental policies against the risk factors of preeclampsia, lack of awareness among healthcare providers and pregnant women about the importance of these trace elements in preventing preeclampsia and other related pregnancy complications.

Assessing these relationships in pregnant women in the prestigious University of Benin Teaching Hospital (UBTH) as a case study is important for several reasons:

1. Preventive Health: Early detection of potential preeclampsia in pregnant women with abnormal levels of trace elements like copper, zinc and selenium can lead to timely interventions helping to prevent the progression of preeclampsia into related pregnancy complications thus aiding to reduce the number of maternal deaths on account of preeclampsia and its related complications.

2. Reproductive Health Relevance: Given the increasing prevalence rates of preeclampsia in pregnancy, understanding its impact on maternal health will provide valuable data to inform reproductive health policies aimed at preventing preeclampsia in pregnant women, reduces the prevalence of preeclampsia in pregnancy especially in high-risk regions such as in rural areas.

3. Contribution to Existing Literature: There is limited research on the correlation between trace elements like copper, zinc and selenium and pregnancy related complications such as preeclampsia in pregnancy. This study will fill a gap in the current body of knowledge and contribute to understanding how these trace elements impact maternal health in pregnant women.

4. Targeted Interventions: By identifying pregnant women at risk of developing preeclampsia, the study findings will help health care workers in maternal health to understand the pathogenesis of preeclampsia, its risk factors and implication in maternal health , the roles of trace elements like copper, zinc and selenium in the prevention of preeclampsia, raise the need for tertiary health institutions and other antenatal care services to put in place cheap and time effective routine diagnostic assays to assess the levels of these trace elements for the early diagnosis of preeclampsia.

This study is therefore justified as it seeks to address a critical area of reproductive health concern with the potential of improving the overall maternal and fetal outcomes for pregnant women.

1.3 Aim of the Study

The aim of the study is to assess the relationship between preeclampsia and trace elements such as copper, zinc and selenium among pregnant women with or without preeclampsia at the prestigious University of Benin Teaching Hospital (UBTH), Benin City, Edo State, Nigeria. The study seeks to determine whether variations in copper, zinc and selenium homeostasis correlates and is associated with preeclampsia development and to identify causes associated with their abnormal levels in this population.

1.4 Specific Objectives of the Study

1. To measure the levels of copper, zinc and selenium ions of the study participants.

2. To identify study participants who are at risk of developing preeclampsia or have preeclampsia from those who are healthy or are not at risk of developing preeclampsia.

3. To assess the correlation between preeclampsia and changes in the levels of copper, zinc and selenium among study participants.

4. To determine the prevalence of preeclampsia among study participants at risk or have preeclampsia at UBTH.

5. To identify any significant difference in the above-mentioned trace elements homeostasis among the study participants based on their number of gestations.

preeclampsia in pregnancy.

1.5 Scope of Study

This study is limited to the evaluation of serum concentrations of Copper, Zinc and Selenium ions in pregnant women with or without preeclampsia who attend antenatal care at a selected tertiary health clinic in Benin City, Edo State. The study focuses on:

- Population: Pregnant women aged 18 years and above, receiving antenatal care in the clinic.
- Study groups: Participants will be classified into two groups- those diagnosed with preeclampsia and those without preeclampsia (apparently healthy pregnant controls).
- Variables or Parameters assessed: Serum levels of Copper, Zinc and Selenium ions.
- Analytical method: Trace element estimation will be carried out using Atomic Absorption Spectrometry (AAS).
- Geographical scope: Restricted to Benin City, Edo State.
- Time Frame: Data collection and analysis will be carried out within the specified research period of the study.

  The study does not cover other trace elements or minerals outside Copper, Zinc and Selenium nor does it extend to non- pregnant women or pregnant women receiving care in private or secondary health facilities outside the selected tertiary clinic.

1.6 Research Hypotheses

1. Null Hypothesis (H_o): There is no significant correlation between preeclampsia and changes in copper levels among pregnant women at UBTH.

Alternative Hypothesis (H_1): There is a significant correlation between preeclampsia and changes in copper levels among pregnant women at UBTH.

2. Null Hypothesis (H_o): There is no significant correlation between preeclampsia and changes in zinc levels among pregnant women at UBTH.

Alternative Hypothesis (H_1): There is a significant correlation between preeclampsia and changes in zinc levels among pregnant women at UBTH.

3. Null Hypothesis (H_o): There is no significant correlation between preeclampsia and changes in selenium levels among pregnant women at UBTH.

Alternative Hypothesis (H_1): There is a significant correlation between preeclampsia and changes in selenium levels among pregnant women at UBTH.

4. Null Hypothesis (H_o): There is no significance difference in changes in copper, zinc and selenium homeostasis among pregnant women based on their number of gestations.

Alternative Hypothesis (H_1): There is a significance difference in changes in copper, zinc and selenium homeostasis among pregnant women based on their number of gestations.

5. Null Hypothesis (H_o): The levels of copper, zinc and selenium among the pregnant women do not follow a normal distribution.

Alternative Hypothesis (H_1): The levels of copper, zinc and selenium among the pregnant women follow a normal distribution.

6. Null Hypothesis (H_o): The prevalence of preeclampsia among pregnant women at UBTH follow a normal distribution.

Alternative Hypothesis (H_1): The prevalence of preeclampsia among pregnant women at UBTH does not follow a normal distribution.

1.7 Research Questions

1. What are the levels of copper, zinc and selenium among study participants and do disturbances in these levels in the study population increase the risk of developing preeclampsia?

2. Is the number of study participants who are at risk of developing preeclampsia or have preeclampsia

3. Is there a significant correlation between preeclampsia and changes in the level of copper, zinc and selenium in the study population?

4. What is the prevalence of preeclampsia among study participants at risk or have preeclampsia in UBTH?

5. Is there an association between the number of gestations and an increased risk of preeclampsia in the study population?

6. Do an increase or decrease in either the levels of copper, zinc and selenium induces antagonistic effects against each other even when they are within daily reference range thus affecting their potency as promising biomarkers in predicting preeclampsia in the study population?

CHAPTER TWO

LITERATURE REVIEW

2.1 Preeclampsia

Preeclampsia is seen as the onset of hypertension after the 20th week of gestation along with evidence of maternal organ failure such as renal insufficiency, hematological complications (Turbeville & Sasser, 2020).

According to Nirupama et al. (2021), Preeclampsia is seen as a pregnancy related multisystem disorder with frequently encountered pregnancy related medical complications next to gestational diabetes. It can be said that preeclampsia is a disease of the placenta since one of its etiological factors is placental insufficiency.

Preeclampsia as a pregnancy related disorder is of two types based on their severity which include Placental Preeclampsia and Maternal Preeclampsia with placental preeclampsia being more severe.

In a developing country like Nigeria, the prevalence of preeclampsia is steadily increasing at a rate of 2-17% among pregnant women with approximately 15% of maternal death being reported annually (Olaoye et al., 2019).

2.1.1 Pathogenesis

Pregnancy results in temporary physiological adaptations that can lead to harmful complications such as preeclampsia (Turbeville & Strasser, 2020).

The pathogenesis and etiology of preeclampsia is said to be multifactorial with acknowledged contribution by placental, vascular, renal and immunological dysfunctions.

The pathogenesis of preeclampsia is complex and not fully understood but it is generally accepted that it results from abnormal placentation, maternal immune maladaptation and systemic endothelial dysfunctions.

In normal pregnancy, normal placentation occurs when the trophoblast cells invade the maternal spiral arteries to form high-capacity low resistance blood vessels.

In preeclampsia, abnormal placentation is due to shallow and incomplete trophoblastic invasion leading to poor remodeling of maternal spiral arteries, reduced utero-placental blood flow and placental ischemia-reperfusion, placental hypoxia among others. The effects of placental ischemia- reperfusion and placental hypoxia are that they cause the release of antiangiogenic factors into maternal circulation. These antiangiogenic factors such as soluble fms- like tyrosine kinases-1 (sFlt-1), soluble endoglin (sEng) binds and neutralizes vascular endothelial growth factor (VEGF), placental growth factor (PIGF), inhibits TGF-β signaling reducing angiogenesis and impairing endothelial function respectively leading to systemic endothelial dysfunctions, angiogenic and vasoconstrictors (endothelin, thromboxane) and vasodilators (nitric oxide, prostacyclin) imbalances.

These systemic dysfunctions and imbalances causes increased vascular permeability leading to edema and proteinuria due to an increased in the flow of body fluids (blood, lymphatic fluid),

oxidative stress, increased vasoconstriction leading to hypertension, activation of coagulation cascade and platelet aggregation as a result of changes in the level of vasodilators such as endothelin, nitric oxide which increase the level of calcium (Ca^{2+})ions in the blood, an electrolyte involved in blood coagulation and blood flow regulation leading to blood coagulation disorders which result to complications such as organs failure, atherosclerosis among others thus preeclampsia is seen as a complication of maternal cardiovascular and renal diseases.

In preeclampsia, immune maladaptation occurs due to maternal immune system responding abnormally to paternal antigens in the fetus leading to impaired placentation as a result of inadequate maternal tolerance to trophoblastic cells, maternal immune cells imbalance, increased level of pro-inflammatory cytokines such as TNF-α, IL-6 which further damage the endothelium and cause persistent inflammation as a result of an increase in the release and levels of acute phase reactants such as C- reactive protein.

Generally, the severity of the pathological effects of preeclampsia depends on genetic, environmental, lifestyle and maternal risk factors such as genetic predisposition (family history of preeclampsia), preexisting medical conditions (chronic hypertension, gestational diabetes, obesity, renal diseases and liver diseases), lifestyle (medications, chronic alcohol consumption, smoking, increased consumption of salty diets), multiple pregnancy, advanced maternal age, parity.

2.1.2 Clinical Signs and Symptoms

The above pathogenesis mechanisms of preeclampsia lead to its hallmark features of hypertension, oxidative stress, proteinuria, edema, end organ dysfunction (liver, renal, brain, coagulation and immunological disorders).

Here, preeclampsia is featured by a persistent elevated systolic / diastolic blood pressure of ≥ 140/ 90 mmHg, oxidative stress as well as proteinuria of ≥ 300 mg / 24-hour urine after 20th weeks of gestation leading to edema, renal dysfunction among others which eventually manifests as uteroplacental complications such as intrauterine growth restrictions, miscarriages/ premature stillbirths, preterm birth, fetal cognitive impairments as well as others.

2.1.3 Diagnosis of Preeclampsia

Preeclampsia is generally diagnosed as new- onset hypertension after 20 weeks of gestation accompanied by proteinuria and signs of maternal organs dysfunction or uteroplacental abnormalities.

The diagnostic criteria of preeclampsia include hypertension, proteinuria, maternal organs dysfunction, fetal complications.

Hypertension as a diagnostic criteria involves use of screening tools such as the regular measurement of blood pressure using a blood pressure cuff / monitor or a sphygmomanometer which diagnose preeclampsia as elevated rise in systolic and diastolic blood pressure ≥ 140 / 90 mmHg respectively on two occasions at least 4 hours apart after 20 weeks of gestation or sometimes as severe hypertension with an elevated rise in systolic blood pressure ≥ 160 mmHg or diastolic blood pressure ≥ 110mmHg confirmed within minutes.

Proteinuria is a classical marker in the diagnosis of preeclampsia. Here, preeclampsia is diagnosed as concentration of plasma proteins such as albumin in urine being ≥ 300 mg / 24-hour urine collection, urinary protein/ albumin- creatinine ratio (ACR) ≥ 0.3 or an urine dipstick reading ≥ or equal + 1

The diagnosis of preeclampsia can also be made with evidences of maternal organs dysfunction such as:

- Renal insufficiency: serum creatinine levels > 1.1 mg/ dL (97.2 μmol/L) indicates preeclampsia.
- Liver dysfunction: elevated rise in liver enzymes such as liver transaminases (ALT, AST, GGT), Lactate Dehydrogenase (LDH) with epigastric pain indicates preeclampsia.
- Neurological complications: headache, visual disturbances, eclamptic seizures indicates preeclampsia.
- Hematological complications: thrombocytopenia/ decreased platelets count (< 100,000/ μL), hemolysis, disseminated intravascular coagulation (DIC) indicates preeclampsia.
- Edema: swollen limbs, feet, face indicates preeclampsia.
- Persistent inflammation indicates preeclampsia.
- Fetal complications such as fetal growth restriction, abnormal Doppler findings of uteroplacental blood flow, oligohydramnios among others indicate preeclampsia.
- Preeclampsia can also be diagnosed using ancillary confirmatory tests such as urine proteins measurement, serum creatinine estimation, use of biomarkers such as placental growth factors, CRP, copeptin, pregnancy associated plasma protein A, trace metals and metals estimation (Ca, Mg, Zn, Cu, Se) using colourimetric, spectrophotometric and immunoassays techniques.

The use of placental growth factors and PIGF to sFlt- 1 ratio in the diagnosis of preeclampsia helps to differentiate preeclampsia from other pregnancy related complications.

2.1.4 Treatment / Management of Preeclampsia

Generally, Preeclampsia has no definitive cure thus its treatment is limited to symptomatic management and early delivery of the placenta and fetus.

The management of preeclampsia focuses on preventing maternal and fetal complications, deciding the optimal timing of delivery against prolonging pregnancy safely.

In the management of mild preeclampsia (BP ≥ 140/ 90 mmHg), When the gestational age is < 37 weeks and mother / fetus is stable, the expectant management to be considered include bed rest and reduced activity, regular monitoring of blood pressure, urine protein , liver / renal function, platelets count , fetal monitoring/ ultrasound, antihypertensive and antioxidants medications such as sildenafili citrate, magnesium sulphate, Vitamin E, copper, zinc and selenium supplements respectively that improves fetoplacental blood circulation, reduces the hypoxic conditions of preeclampsia and oxidative stress levels , prevents preeclamptic seizures, use of drugs that promotes angiogenesis, early delivery of fetus at 37 weeks if maternal / fetal condition worsens.

In the management of severe preeclampsia (BP ≥ 160/ 110 mmHg), the expectant management to be considered are hospital admission and close monitoring, antihypertensive medications such as IV hydralazine, IV labetalol, nifedipine, magnesium sulphate to reduce the risk of developing hypertensive complication such as stroke, seizures, anti-inflammatory medications such as corticosteroids, aspirin to reduce the effects of persistent inflammation, sufficient fluid balance to manage edema.

Preeclampsia as a whole can be managed, treated and infact even prevented through lifestyle modifications and regular medical checkup before, during and after pregnancy.

The consequences of a delay in the diagnosis, management and treatment of preeclampsia extend far beyond preterm delivery, it causes lasting effects for both mother and child leading to increased susceptibility to chronic hypertension, cardiovascular disease and renal diseases later in life.

In summary, preeclampsia is one of the leading causes of maternal mortality and morbidity, neonatal and fetal mortality mostly due to preterm delivery, placental abruption, intrauterine growth restrictions.

2.2 Antioxidants

The unique feature of the pathogenesis of preeclampsia is the disturbances of extra villous trophoblast migration towards the uterine spinal arteries which cause increase in uteroplacental vascular resistance, vascular dysfunction, platelet adhesion and aggregation resulting in reduced systemic vasodilatory properties, intravascular coagulopathy, placental infarction, impaired uteroplacental blood flow. This underlying pathogenesis appeared to be an altered bioavailability of nitric oxide and tissue damage due to an increase in the levels of reactive oxidative species (ROS) and reactive nitrogen species (RNS). Their increase is due to the condition known as oxidative stress which decreases the mechanisms of the maternal antioxidant system.

Oxidative stress is seen as the imbalance between pro-oxidants and antioxidants levels and production in favour of the oxidants. The consequence of oxidative stress in preeclampsia is that it results in deficiencies in oxygen and nutrients required for normal fetal development leading to fetal growth restriction (Taysi et al., 2019).

The maternal antioxidant system comprises of defense mechanisms that prevent free radical damages to body cells. These defense mechanisms prevent the reactions of free radicals such as

ROS, super oxides with macro molecules like lipids, slow down free radical and non- radical redox reactions such as lipids peroxidation and repair or eliminate damaged molecules.

The compounds responsible for these defense mechanisms are of endogenous and exogenous origin and based on their mechanism of action, they are classified into enzymatic and non-enzymatic antioxidants. These antioxidants scavenge ROS and protect tissues against oxidative damage.

Enzymatic antioxidants include catalase, superoxide dismutase (SOD), iodothyronine deiodinase, selenozymes, thioredoxin reductase, glutathione peroxidase (GSH-Px) among others which protect the vasculature from ROS, maintaining vascular function.

The non- enzymatic antioxidants include vitamins (Vit .C,Vit.E), trace elements, melatonin, phenolic compounds, ceruloplasmin, glutathione, flavonoids.

In preeclampsia, the pathogenesis of placental ischemia reduces antioxidant activity thus the extent of oxidative stress increases and lead to the development of the pathological findings of preeclampsia such as proteinuria and hypertension (Taysi et al., 2019).

2.3 Essential Trace metals / Essential Trace elements

Essential trace elements are micronutrients which are catalytic substances needed by the human body in small amount in form of metallic ions (Otebhi & Osadolor, 2019). These metallic ions cannot be synthesized by the body thus are supplemented or sourced from diet. They are needed for critical body functions such as growth, development, homeostasis, metabolism, reproduction, immunity among others.

Essential trace elements with known physiological activities in the body include iron (Fe), copper (Cu), zinc (Zn), selenium (Se), manganese (Mn), cobalt (Co), chromium (Cr) among other essential trace elements. Their physiological activities are as a result of their interaction with organic molecules present in the body.

Trace elements in form of their metallic ions are found in the body system in form of conjugated compounds such as complex salts, metalloproteins and other chelating compounds. Here, most essential trace metals that exist as metalloproteins are involved in the body metabolic pathways as being components and cofactors of enzymes, they make use of transport plasma proteins such as albumin, transferrin, ceruloplasmin to reach their target sites.

Changes in the levels of essential trace metals in the body tend to lead to deficiencies and toxicities of these metals resulting in a series of deleterious physiological and metabolic disorders and dysfunctions clinically manifested as impaired biochemical reactions, clinical diseases, cognitive disorders, free radical damage to DNA and cell membranes affecting intracellular enzyme activity, metabolic effects, antioxidant activity, gene expression and regulation, immunological function. These levels are expressed as either mg/dL /ng/ dL/ µg/dL.

The deficiency and toxicity of these essential trace elements is due to dysregulations and disorders of the body homeostatic mechanisms used by the body to keep their levels in equilibrium. This homeostatic regulation involves the processes of absorption, metabolism, storage and excretion and the relative importance of these processes varies among each essential trace elements.

The main controlling mechanism here is the regulation of the amount of trace elements absorbed from the gastrointestinal tract, excretion of excess amounts into the urine, faeces, bile, sweat and

storage of trace elements in inactive physiological forms such as copper as metallothiolein, iron as ferritin so as to prevent the deleterious effects of deficiency and toxicity of essential trace metals.

In pregnancy, essential trace elements are critical for healthy fetal growth, maternal well- being, normal functioning of enzymes, hormones and antioxidant systems since pregnancy itself as a reproductive process involves a series of regulated physiological and biochemical changes.

Each essential trace element has its specific role in pregnancy critical for fetal and maternal health. The key roles of essential trace elements here are enzyme activity, antioxidant defense, immune function, fetal development.

In enzyme activity, essential trace metals are involved in catalytic processes such as being activators of regulatory, metabolic and antioxidant enzymes, attract and facilitates the conversion of substrate molecules to specific end products, act as integral constituents of numerous regulatory enzymes involved in cell division, differentiation and fetal development.

In antioxidant defense, essential trace metals act as electron acceptors and donors in redox reactions depending on the specific metal and its role in the process. Most trace metals that play an antioxidant role during pregnancy act mainly as electron donors so as to neutralize the generation of free radicals thus crucial in preventing oxidative stress and cellular damage during pregnancy.

In immune function, essential trace elements are important for both the maternal and fetal immune response to infections, diseases and changes in the internal and external environment.

In fetal development, essential trace elements are vital for cell proliferation and differentiation, healthy embryonic growth and development, nucleic acids synthesis, gene expression.

In conclusion, maternal deficiency and toxicity of these trace elements during pregnancy as a result of imbalance and changes in their levels in the body can lead to pregnancy related complications such as miscarriage, low birth weight, premature birth, intrauterine growth restriction, preeclampsia, congenital anomalies (birth defects) among others.

2.3.1 Copper (Cu)

Copper is an essential trace element with an atomic number of 29 with atomic weight of about 63.5 in group 11 of the periodic table. It is a vital trace element present in the body in the form of organic complexes such as metalloproteins and its oxidized ionic forms consist of cuprous ion (Cu^{+}) and cupric ion (Cu^{2+}). Most of the levels of copper in the body are concentrated in the bones, muscles, hair, nails, blood serum and plasma, urine, brain, liver and kidneys

Copper is physiologically active in its oxidized cupric ionic form (Cu^{2+}) due to its stable solubility in the blood and other body fluids, ability to exist in variable oxidation states and acts as both an electron acceptor and donor thus its main physiological and biochemical function as acting as an antioxidant and scavenger of free radicals.

Copper is essential as a metallic cofactor of several enzymes known as cuproenzymes such as cytochrome c oxidase, monoamine oxidase, dopamine β- hydroxylase , tyrosinase, lysyl oxidase, ferroxidases, superoxide dismutase, ceruloplasmin among others involved in energy production, neurotransmission through formation and maintenance of myelin sheath and neurotransmitter metabolism of serotonin, dopamine and norepinephrine, melanin formation, connective tissue

formation, iron metabolism and erythropoiesis, antioxidant function and scavenging of free radicals respectively.

The average copper level in the body is approximately 100-200 mg with a plasma concentration of 70- 140 µg / dL (Yin et al., 2025).

In pregnancy, the recommended daily intake of copper is about 1-3 mg per day for pregnant mothers.

2.3.1.1 Dietary Sources of Copper

According to Yin et al. (2025), dietary consumption serves as the main source of copper in the body include diet (most abundant in organs meat, wheat, nuts, chicken, seafood, legumes), water consumption and dietary supplements in form of copper amino acids chelates, copper sulphates, copper gluconate.

2.3.1.2 Absorption, Transport, Metabolism and Excretion of Copper

The body maintains a stringent copper metabolism mechanism to oversee the absorption, utilization, storage and elimination of copper.

In copper absorption, the dietary amount of copper absorbed from the gastrointestinal tract is proportional to the amount of copper in the diet.

Dietary factors influence copper absorption and metabolism leading to changes in the level of copper in the body thus dietary factors are seen as a homeostatic mechanism used in the regulation of copper levels in the body to prevent the effects of copper toxicity and deficiency. The levels of copper in the body are regulated by a sophisticated system to keep them within the appropriate range preventing imbalances from adversely affecting health.

The absorption of copper is affected by the presence of other substances which are either enhancers or inhibitors of copper absorption. Substances such as phytates, phosphates, iron, zinc, amino acid histidine, heavy metals like cadmium, molybdenum, mercury, lead, nickel, arsenal tends to slow down or inhibits copper absorption.

Dietary copper absorption mainly occurs in the duodenum and jejunum. Here, Cu^{2+} is converted to Cu^{+} by transmembraneous proteins after being absorbed by copper transport proteins of the intestinal mucosa which is then transported through the enterohepatic circulation to the liver bound to albumin.

In the liver, copper is metabolized, processed and then bounded to proteins such as ceruloplasmin, superoxide dismutase, metallothionein so as to be distributed and utilized by the body tissues.

Copper is mainly distributed between the blood plasma and red blood cells. In the blood plasma, 93% of copper are bounded to the protein ceruloplasmin with 7 % of the remaining copper less firmly bounded to plasma albumin. In the red blood cells, 60% of copper exists as zinc -copper metalloenzyme superoxide dismutase with the remaining 40% of copper loosely bound to proteins.

Most endogenous copper is excreted in the gastrointestinal tract (GIT) through the bile into faeces, with only a small amount of biliary copper being reabsorbed. Little amount of copper is lost through the urine and sweat (Yin et al., 2025).

2.3.1.3 Deficiency of Copper

Insufficient intake of copper leads to the depletion of liver copper reserves, and thus copper deficiency with resultant disease, tissue / organ injury and damage, and in extreme cases, death can occur.

The most common clinical signs and symptoms of copper deficiency include hypochromic anaemia, fatigue, decreased number of white blood cells leading to increased susceptibility to infections, abnormal bone formation with skeletal fragility and osteoporosis, neurological damages, impaired growth and development, metabolic abnormalities, cardiovascular damage.

Copper deficiency is as a result of intestinal malabsorption, gastrointestinal surgery, nutritional deficiency, Menkes disease / occipital horn syndrome due to mutations in copper transport proteins causing impaired copper transport.

Copper deficiency is usually treated and managed through the sufficient copper rich diet and supplements, treatment of underlying effects and causes of copper deficiency.

2.3.1.4 Toxicity of Copper

Copper toxicity rarely occurs but if it occurs is as a result of excessive copper consumption, dysregulation of copper homeostatic mechanisms with the severity of copper transport dependant on the dosage and exposure of excess copper.

Individuals with genetic disorders and diseases affecting copper metabolism (liver cirrhosis, Wilson disease, copper toxicosis) are at a high risk of developing chronic copper toxicity even with significant low intake of copper.

2.3.1.5 Relationship between Preeclampsia and Copper Homeostasis

Copper ions is important in pregnancy for physiological and biochemical processes such as erythropoiesis, collagen, melanin, and myelin synthesis, regulation of redox reactions, iron metabolism and transport, fatty acids metabolism (Grieger et al.,2019).

In pregnancy, Cu as a metal with high redox potential acts as a cofactor for different proteins involved in numerous reactions such as iron metabolism, free radical elimination, aerobic respiration for energy production, connective tissue formation, normal neurological function, normal cell function, neural myelination, neurotransmitters metabolism, regulation of other metals levels in the maternal body system such as zinc (Jagodic et al., 2022).

Copper ions in the maternal blood plasma are known for their antioxidant and anti-inflammatory roles (Grzeszczak et al., 2020). They are known to influence enzymatic activities, regulate gene expression and synthesize proteins in pregnancy (Grzeszczak et al., 2020).

In pregnancy, copper ions are needed for fetal growth and development, angiogenesis, oxidative stress control. Thus, they reduce the risk of complications such as hypochromic anemia, pregnancy induced hypertension, low birth weight, miscarriage, postnatal complications.

Deficiency of copper during pregnancy is usually as a result of insufficient maternal dietary intake of copper which tends to lead to an increased risk of giving birth to low birth weight neonates, developing hypochromic anaemia, intrauterine growth restriction, spontaneous abortion, decreased fertilization rates and oocyte recovery rate (Jagodic et al., 2022).

Toxicity of copper in pregnancy may lead to Wilson disease, liver cirrhosis, Fenton reaction (formation of reactive oxidative species, hydroxyl radicals that damages fetal DNA, proteins and

lipids through the promotion of oxidative stress and peroxidation), pre-term births, preeclampsia, low birth weight, postnatal complications such as gestational diabetes.

Thus, the toxicity and deficiency of copper ions in the maternal blood plasma or altered copper homeostasis is associated with pregnancy induced complications such as preeclampsia.

According to some studies, hypercupremia (elevated serum copper level) is implicated in the pathogenesis of preeclampsia since it promotes free radical generation through fenton reactions, influences inflammation pathways involved in preeclampsia leading to lipids peroxidation and endothelial damage.

2.3.2 Zinc (Zn)

Zinc is an essential trace element with symbol Zn and atomic number 39 with an atomic weight of 65.4. It is the second most abundant metal after iron which is present in the body in form of metalloproteins and its divalent ionic form of Zn^{2+} with most of the levels of zinc highly concentrated in the muscles, bones, skin, hair, nails, liver, pancreas, brain and kidneys.

Zinc as an essential trace metal is involved in various physiological and biochemical processes in the body due to it not being redox active. It is critical for growth and development, immune function due to its antimicrobial properties, enzymatic activities as a cofactor for enzymes involved in body metabolism, cell growth and antioxidant defense.

Zinc is involved in the synthesis and folding of body proteins, energy and lipids metabolism, plays an important role in cell division, DNA synthesis, genetic expression and regulation, activation and regulation in the secretion of hormones like insulin, testosterone, oestrogen, gametogenesis (spermatogenesis and oogenesis), normal brain and neurological functioning, cell signaling, wound healing among others.

The average plasma concentration of zinc in the body is about 70- 120 µg/ Dl.

In pregnancy, the recommended daily intake of zinc for pregnant mothers is about 19 mg per day.

2.3.2.1 Dietary Sources of Zinc

Zinc is found in a wide variety of foods such as wheat, dairy products, chicken, red meat, milk, eggs, liver, cheese, fish, seafood products, nuts, cereals, legumes, spices, dietary zinc supplements in form of zinc amino acids chelates, zinc sulphate, zinc gluconate, zinc acetate.

2.3.2.2 Absorption, Transport, Metabolism and Excretion of Zinc

Zinc homeostasis is tightly regulated so as to prevent zinc toxicity and deficiency leading to a variety of disorders. The homeostatic mechanism of zinc oversees the absorption, transport, metabolism, utilization, storage and elimination of zinc in the body.

Zinc absorption takes place in the duodenum and small intestine. Zinc is easily absorbed from dietary sources of animal origin like meat, egg, seafood since they contain sulphur containing amino acids (methionine and cysteine) that enhances intestinal zinc absorption and zinc bioavailability and relative absent compounds (phyates, oxalates, phosphates, heavy metals) that inhibits intestinal zinc absorption.

The divalent metal transporter 1 protein (DCT 1) is responsible for the transport of zinc into the enterocyte. This transport protein alongside with Zn transporter (ZnT) and Zrt-, Irt- related proteins (ZIP) contribute to a wide array of zinc physiological, biochemical and cellular functions (such as immune, endocrine, reproductive, skeletal and neuronal) and the tight regulation of its homeostasis. DCT 1 present in the intestinal brush border is capable of binding

not only to zinc divalent ions but also other elements such as iron, copper, calcium, magnesium, heavy metals. Thus, zinc bioavailability is affected by the competitive binding and interaction of DCT 1 between zinc and other divalent ions in the gastrointestinal tract.

From the intestinal lumen, zinc ions bind to metallothionine orare stored in secretory vesicles. Here zinc cations form stable complexes with proteins. The plasma/ serum zinc pool consists of about 80% of zinc ions that are loosely bounded to albumin and 20% of zinc ions tightly bounded to α2- macroglobulin.

About approximately 2- 60% of zinc is stored in body tissues such as skeletal muscles, bones, liver, skin, hair, nails, body fluids such as blood plasma and serum, urine, sweat.

The release of zinc when in excess is facilitated by GIT secretion, increased renal excretion while zinc reabsorption is greatly increased during dietary zinc limitation.

2.3.2.3 Zinc Deficiency

Zinc deficiency is associated with insufficient dietary intake of diet rich in zinc, depletion of zinc reserves due to increased physiological demands as in states such as in pregnancy, lactation, stress, obesity, increased physical growth, impaired zinc transport due to genetic mutations of zinc transport proteins, decreased plasma albumin levels. Impaired intestinal zinc absorption is as a result of diarrhea, malabsorption syndromes, alcoholism induced lack of appetite, medications such as diuretics, contraceptives, folates and chronic renal failure.

The numerous clinical symptoms of zinc deficiency include growth retardation, hypogonadism, delayed sexual maturation, cognitive impairment and behavioural abnormalities, increase in susceptibility to infections, delayed or slow wound healing among others.

2.3.2.4 Zinc Toxicity

The deleterious effects here include fatigue, nausea and vomiting, abdominal cramps, anaemia, impaired immune function leading to leucopenia, neurodegenerative diseases, insomnia, memory loss among others.

2.3.2.5 Relationship between Preeclampsia and Zinc Homeostasis

Zinc ions in the maternal blood plasma are known for their antioxidant and anti-inflammatory roles. They are known to influence enzymatic activities, regulate gene expression and synthesize proteins and DNA in pregnancy.

In pregnancy, zinc ions are needed for fetal growth and development. Thus, they reduce the risk of complications such as hypochromic anemia, pregnancy induced hypertension, low birth weight, postnatal complications.

Zinc ions in maternal and placental circulation during pregnancy is essential for cell division and differentiation for fetal cells and organs development, fetal bones mineralization, regulation of fetal and maternal homeostasis, ,regulation of the secretion of hormones such as growth hormones, insulin, testosterone, thymulin a thymus hormone needed for immune functions, activation of metalloproteases and antioxidant enzymes such as superoxide dismutase, glutathione peroxidase that eliminates and scavenges free radicals, inhibition of unsaturated fatty acids, antagonisation of heavy metals like cadmium ,lead, detoxification of reactive oxidative species, nitrogenous compounds, organic compounds, embryogenesis, genetic expression and regulation and fetus development.

In pregnancy, zinc reduces the generation of inflammatory cytokines and chemokines like C-reactive protein (CRP), interleukins such as IL -6, monocyte chemo-attractant protein (MCP-1),

secretory cell adhesion molecules. Thus, zinc reduces the incidence of maternal infections, levels of oxidative stress and inflammatory markers such as CRP, adrenocorticosteroids in pregnancy. Zinc is a regulator of NF-kb pathway in maternal and placental immunity thus zinc plays a critical role in maternal and placental immunity, inflammation during pregnancy by regulating the activation of cytokines, chemokines and adhesion molecules such as IL-1β, IL-6, IL-8, TNF-α, CRP, fibrinogen, selectins and integrins (Grzeszczak et al., 2020).

However, the toxicity and deficiency of zinc increases the risk of pregnancy induced complications such as preeclampsia. Studies show that decreased plasma concentration of zinc less than70 µg/dl can lead to hypercupremia due to a greater competitive binding between copper and zinc ions to transport molecules in the placental and maternal circulation thus leading to preeclampsia which is clinically manifested as oxidative stress, impaired vascular function and abnormal immune regulation (Grzeszczak et al., 2020).

According to Kumari et al. (2022), pregnant women are considered a high-risk group for zinc deficiency due to decreased intestinal absorption of zinc during pregnancy, lifestyle factors such as smoking, alcohol abuse, strenuous exercise, medications. Apart from preeclampsia, zinc deficiency in pregnancy can induced prolonged labour, infertility, teratogenesis, impaired placental growth, intrauterine growth restriction, embryonic or fetal death as well as increased anxiety, depression, irritability, stress, impaired immune functions leading to acute phase reactions induced by inflammation and maternal infections.

The deficiency of zinc in pregnancy reduces the activity of thymulin, a hormone responsible for the regulation of placental immunity, induces lymphopenia, inflammation associated vascular

dysfunctions. According to Iqbal et al. (2020), there is a linear association between pregnancy related complications such as preeclampsia and zinc deficiency in maternal circulation.

2.3.3 Selenium (Se)

Selenium is a naturally occurring metalloid element with an atomic number of 34 which is essential to human health in trace amounts since it is toxic to the body system in excess. In nature, selenium exists in both organic and inorganic forms (Genchi et al., 2023).

As an essential trace element, selenium plays a tremendous role in the functioning of the body system as it is used for the biosynthesis of selenoproteins, selenoenzymes involved in body cells antioxidant defense, maintenance of redox homeostasis, immune system functioning, cell proliferation, cell transformation and aging, cell signaling and transduction, control of reproductive function, neuroprotection among others (Minich, 2022).

Selenium is incorporated into selenoproteins thus supports antioxidant defense systems due to its high antioxidant activity. Apart from its antioxidant function, selenium in form of selenoenzymes and selenoproteins is involved in the synthesis of thyroid hormones thus involved in the regulation of the body basal metabolism.

Due to selenium important and unique role in the body, it is the only essential trace element genetically encoded as a constituent of the amino acid selenocysteine. In the form of selenocysteine, selenium is present in the active sites of antioxidant enzymes (glutathione peroxidases, thioredoxin reductases and iodothyronine deiodinases).

According to World Health Organization (WHO), the recommended daily intake of selenium for adults is about 55 µg per day while for pregnant women, The daily requirement is about 60 micrograms per day. The average plasma level of selenium is about 60- 150 ng/mL.

2.3.3.1 Dietary Sources of Selenium

The greatest amount of selenium is found in beef, pork, eggs, poultry, seafood, nuts, organ meat, cereals, vegetables, onions, mushrooms, fruits like tomatoes and sometime seen in dietary selenium supplements.

2.3.3.2 Absorption, Transport, Metabolism and Excretion of Selenium

About 50- 90% of dietary selenium is well absorbed from the digestive tract. Dietary selenium is mainly bounded to the amino acids selenocysteine and selenomethionine.

Inorganic selenium when absorbed into the body, is initially bound to erythrocytes, albumin and plasma globulins, and then is transported into body tissues.

In the blood plasma, liver, spleen, muscles, Selenium in its inorganic forms form complexes with proteins. In pregnancy, these complexes can easily cross the blood-placenta barrier to reach the fetus for fetal growth and development.

The most absorbed forms of selenium are stored in the thyroid gland, liver, pancreas, kidneys, pituitary glands as well as in hair and nails.

The half-life of selenium after its physiological activity is 65- 115 days. About 60% of selenium is eliminated from the body in form of urine while about 30% is eliminated in form of faeces, sweat.

2.3.3.3 Selenium Deficiency

The insufficient dietary intake of selenium usually results to clinically endemic disorders such as Keshan and Kashin –Beck disease, muscle necrosis, cardiomyopathy, myocardial infarction cardio-cerebrovascular disease, male infertility, hypothyroidism, increased risk of cancer,

immune system abnormalities leading to increased susceptibility to infections, respiratory distress syndrome, metabolic disorders (type 2 diabetes mellitus and dyslipidaemia), liver diseases, chronic renal failure among others.

2.3.3.4 Selenium Toxicity

Selenium toxicity or poisoning (selenosis) is as a result of excess dietary selenium intake which imposes risks and damage on human health.

Selenium toxicity causes the inhibition of cell proliferation, DNA replication, proteins synthesis and induces lipids peroxidation clinically manifested as garlicky breath, dermatitis, hair and fingernail loss among others.

2.3.3.5 Relationship between Preeclampsia and Selenium Homeostasis

In pregnancy, Selenium is an essential trace element that is important for ovarian follicular growth and maturation, parturition, implantation (Grieger et al., 2019). Selenium in pregnancy is needed for the regulation of thyroid hormone metabolism, calcium metabolism and synthesis of DNA (Kumari et al., 2022).

Also in pregnancy, selenium affects and influences uterine function, embryonic development, conceptous growth, gene and angiogenic factors expression, placental and fetal growth and development, improvement of fetal substrate supply, regulation of production of pro-inflammatory proteins during labour and conception.

Selenium in pregnancy is an important antioxidant since it is a component of antioxidant enzymes like selenoproteins (S, P, W), iodothyronine deiodinases, glutathione peroxidases (GPX), thioredoxin reductases (TRXR) which reduces the generation of reactive oxidative

species thus prevents fetal DNA damage and protects against oxidative stress (Dahlen et al., 2022).

According to Duntas (2020) examined, selenium deficiency in pregnancy has been associated with adverse pregnancy outcomes like miscarriages, neural tube defects, pre-term births, low birth weight, retarded fetus intrauterine growth, preeclampsia, gestational diabetes, hernia.

Here, selenium deficiency in the 2^{nd} and 3^{rd} trimester of pregnancy increases the generation of reactive oxidative species due to reduced GPX activity, hypercupremia, increased oxidative stress causing the hypertensive and placental dysfunction disorders of preeclampsia that account for 15% of maternal deaths and preterm births.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The study was conducted at the University of Benin Teaching Hospital (UBTH), a major tertiary health facility in Benin City, Edo State, Nigeria for the collection of blood samples of relevant sample size from pregnant women receiving antenatal care.

3.2 Study Design

The study made use of a hospital based, case control and analytical study design to assess the relationship between preeclampsia and trace elements like copper, zinc and selenium among pregnant women at UBTH. Here, the pregnant women were divided into two groups: (1) Pregnant women diagnosed with preeclampsia with their blood samples labeled “P” and (2) Normotensive pregnant women (controls) whose blood samples were labeled “N”.

Both groups were also matched by gestational age (+- 2 weeks) when possible.

3.3 Study Population

The study involved pregnant women with or without preeclampsia with the participants selected using purposive non-probability sampling technique. Here, the inclusion and exclusion criteria of the participants were considered.

The inclusion criteria included:

- Pregnant women ≥ 20 weeks gestation (especially those in their second and third trimester in which preeclampsia is commonly diagnosed).
- Age 18-45 years with signed inform consent

- The participants were then categorized into a preeclampsia group (those diagnosed with preeclampsia based on clinical criteria such as blood pressure≥ 140/90 mmHg, proteinuria ≥ 300mg/24 hrs) and control group (normotensive pregnant women with no signs on preeclampsia or other hypertensive disorders, history of chronic hypertension, diabetes, renal disease or multiple pregnancy).

  The exclusion criteria included:

- The study excludes pregnant women with chronic illnesses such as diabetes mellitus, chronic hypertension before pregnancy, autoimmune disorders, on trace minerals or antioxidants supplements, those with refused informed consent, multiple pregnancies.
- Pregnant women who are current smokers, heavy drinkers or have been diagnosed with occupational heavy metal exposure.

3.4 Sample Size

The sample size or number of participants was determined using the two- sample t- test sample size formula:

$$n = \frac{2\times(Z\beta+Z\alpha/2)2\times\sigma2}{d2}$$

Where:

- n = sample size group
- Zβ= Standard normal deviate at 80% or 0.84
- $Z\propto/2$= Standard normal deviate at significance level α = 0.05 or 1.96
- $\sigma =$ estimated standard deviation of outcome (from literature)
- $d =$ minimum difference in means to effect size. Here, the sample size was determined by:

$$n = \frac{2\times(0.84+1.96)2\times\sigma 2}{d2}$$

$$n = \frac{2\times 7.84\times\sigma 2}{d2}$$

$$n = \frac{15.68\times\sigma 2}{d2}$$

if n = 40 per group then:

$$40 = \frac{15.68\times\sigma 2}{d2}$$

Thus, if the standard deviation (σ) is 1.6 times larger than the minimum difference (d) to be detected, a total of 80 participants were selected.

3.5 Laboratory Analysis

The blood samples obtained from pregnant women attending the antenatal wards of the prestigious University of Benin Teaching Hospital (UBTH) were analyzed for antioxidant trace elements (Cu, Zn, Se) in the analytical laboratory of the Faculty of Science Laboratory Technology, University of Benin, Benin City using the atomic absorption spectrophotometer (AAS).

3.5.1 Principle of Atomic Absorption Spectrometry (AAS)

Atomic Absorption Spectrometry is an analytical technique used to determine the concentration of specific metal elements (such as electrolytes like calcium, magnesium, sodium, potassium, trace metals like copper, zinc, selenium, nickel, manganese, heavy toxic metals like lead, mercury, aluminum, arsenal among others).

Its principle is based on the absorption of light by free atoms in the ground state implying that ground state atoms absorb light of a specific wavelength with the amount of light absorbed directly proportional to the concentration of the element present in the sample.

Its instrumentation and working principle are based on the concept of Beer Lambert Law which consists of atomization and nebulization, radiation source, absorption and measurement.

In atomization and nebulization, the atomizer of the atomic absorption spectrophotometer allows for the sample usually in liquid form to be introduced either into a flame, graphite furnace, hydride or cold vapor generator where the metals in the sample is converted into free atoms in the ground state in the form of fine aerosol /vapour through the use of the nebulizer.

The radiation source of the atomic absorption spectrophotometer consists of a hollow cathode lamp (specific to the element being analyzed) emits light at characteristic wavelengths of that element.

In absorption, the radiation/ light passes through the vapourized sample using a monochromator or a filter to select the specific wavelength in which atoms of the specific metal element absorb light energy corresponding to their electronic transition (ground state to excited state).

In measurement, the increase in light intensity is proportional to the concentration of the element in the sample, according to Beer – Lambert law.

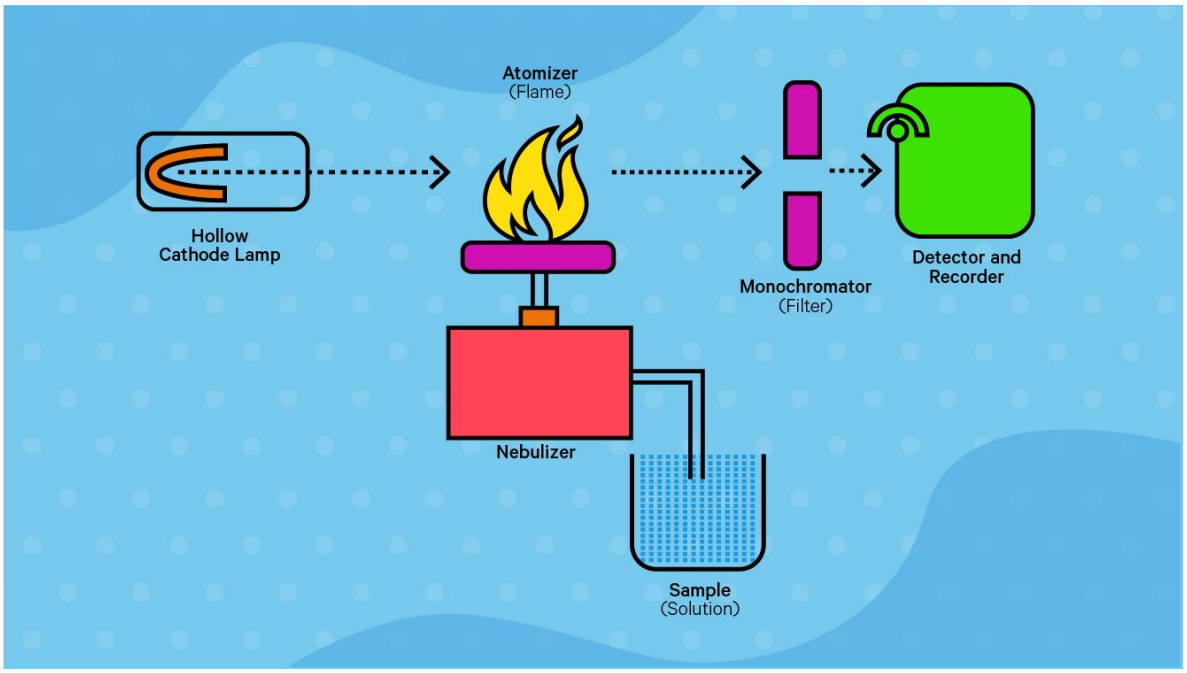


Figure 3.1: Diagrammatic representation of the working principle of the Atomic Absorption Spectrometry (AAS)

3.6 Sample Preparation

Approximately, 5mL of venous blood was drawn aseptically from each participant using a sterile disposable syringe and needle. The blood was collected into clean, leak proof labeled plain sample containers to avoid contamination. Samples were allowed to clot at room temperature for 30- 60 minutes and then centrifuged at 1500 -2000g for 10 minutes to separate the serum. The serum samples were then aliquoted into labeled clean vials and stored at -20ºC until analysis. Here, hemolysis (rupture of red blood cells) was avoided during sample collection.

3.7 Procedure

Sample Dilution

Simple acidic dilution

This method of sample dilution is used in graphite- furnace AAS. It supports the direct analysis of diluted serum. Procedure includes:

3 In a clean environment, 1% v/v ultrapure trace- metal grade nitric acid (HNO_3) was prepared in ultrapure water as a diluent.

4 50- 100 µL of the serum sample was pipetted into an acid cleaned tube with the diluent added in a 1: 10 or 1: 20 dilutions.

5 Internal standards such as indium, rhodium was added at a known concentration to the diluted sample which is then mixed and analyzed.

3.8 Introduction of the Sample into the analyzer of Atomic Absorption Spectrophotometer

1. Nebulization

- Most AAS instruments make use of a nebulizer connected to an aspiration capillary.
- The liquid sample was aspirated by a high – velocity oxidant gas stream (usually air or nitrous oxide) which breaks the liquid into a fine aerosol (tiny droplets).
- Only the fine droplets were carried forward with larger droplet removed by a spray chamber.

2. Transport into the atomizer

The atomization system employed by AAS for Cu, Zn and Se estimation was either the flame AAS, graphite / electrothermal AAS, hydride generation or cold vapor AAS. With the hydride generation AAS used specifically in Se estimation.

a. Flame AAS

- The aerosol entered a flame burner head (commonly air-acetylene or nitrous oxide – acetylene).
- The solvent was evaporated with the trace metals ions in the sample dissociated into atoms in the flame.
- A hollow cathode lamp was used to emits light/ radiation which passes through the flame.
- The radiation emitted was absorbed by the atoms at characteristic wavelength.

b. Graphite Furnace AAS

- A small volume (usually 10-20 μL) of the sample was pipetted into a graphite furnace or tube.
- In the furnace, programmed heating was performed to cause atomization.

c. Hydride Generation AAS

- The sample reacted chemically to form a volatile hydride vapor.
- The vapor was then swept into a heated quartz cell or absorption tube where atoms was formed and absorbed radiation.

d. Optical path and detection

- The beam from the hollow cathode lamp was passed through the atomizer (flame/furnace/ quartz cell).
- The ground state atoms in the light absorbed light at their characteristic wavelength.
- The detector which is a photomultiplier tube measured the intensity of light which is proportional to trace elements (Cu, Zn and Se) concentration in the sample.

3.9 Quality Control

Quality assurance and control in AAS for trace elements (Cu, Zn and Se) estimation is critical for contamination controls. Quality control in AAS was performed by:

- Running method blanks (acidified ultrapure water) to check contamination from reagents/ sample containers.
- Certified reference materials were used to monitor accuracy.
- Recovery tests were run periodically.

3.10 Statistical and Data Analysis

The statistical analysis of the data was carried out using SPSS (Statistical Package for Social Sciences) a statistical software package. Results here are expressed as either continuous variables (serum copper, zinc and selenium levels) or categorical variables (presence or absence of preeclampsia) which was then summarized using mean $\pm$ standard deviation (SD) or mean $\pm$ standard error in mean so as to assess the levels of Cu, Zn and Se ions through their means in the different samples obtained from the same population (pregnant women) .

The mean levels of Copper, Zinc and Se between preeclamptic and non-preeclamptic pregnant women were then compared using independent sample t-test, Student t test, Welsch t test, one – way ANOVA (Analysis of Variance)

Regression analysis was also performed to determine the predictive ability of copper, zinc and selenium levels for the occurrence of preeclampsia (determine if copper, zinc and selenium are dependent variables of preeclampsia).

CHAPTER FOUR

RESULTS

4.0 Results

This study was carried out on a total of 66 participants due to limitations as a result of lack of awareness about the importance of antenatal care among pregnant women in the study population Benin City, time or duration of the study. The participants were grouped into 40 negative non-preeclamptic group and 26 positive preeclamptic group.

The concentrations of selenium (Se), copper (Cu), zinc (Zn) was determined in pregnant women with preeclampsia (n = 26) and normotensive pregnant women (n= 40) with statistical analyses performed using one-way ANOVA (Analysis of Variance) and t tests such as the Welsch t test

The summary of results is presented below.

Table 4.1: Shows the serum concentrations/ levels (µg/dl) of Cu, Zn and Se in pregnant preeclamptic and non-preeclamptic groups.

Samples	Group	Se	Cu	Zn
N40	**Control**	115µg/Dl	69µg/dl	95µg/dl
N39	***C*ontrol**	123µg/Dl	81µg/dl	74µg/dl
N38	**Control**	118µg/Dl	75µg/dl	82µg/dl

N37	**Control**	109µg/Dl	67µg/dl	98µg/dl
N36	**Control**	128µg/Dl	63µg/dl	75µg/dl
N35	**Control**	96µg/dL	54µg/dl	69µg/dl
N34	**Control**	84µg/dl	65µg/dl	95µg/dl
N33	**Control**	90µg/dl	104µg/dl	54µg/dl
N32	**Control**	105µg/dl	113µg/dl	89µg/dl
N31	**Control**	128µg/dl	95µg/dl	76µg/dl
N30	**Control**	143µg/dl	82µg/dl	92µg/dl
N29	**Control**	116µg/dl	75µg/dl	87µg/dl
N28	**Control**	86µg/dl	68µg/dl	65µg/dl
N27	**Control**	95µg/dl	82µg/dl	73µg/dl

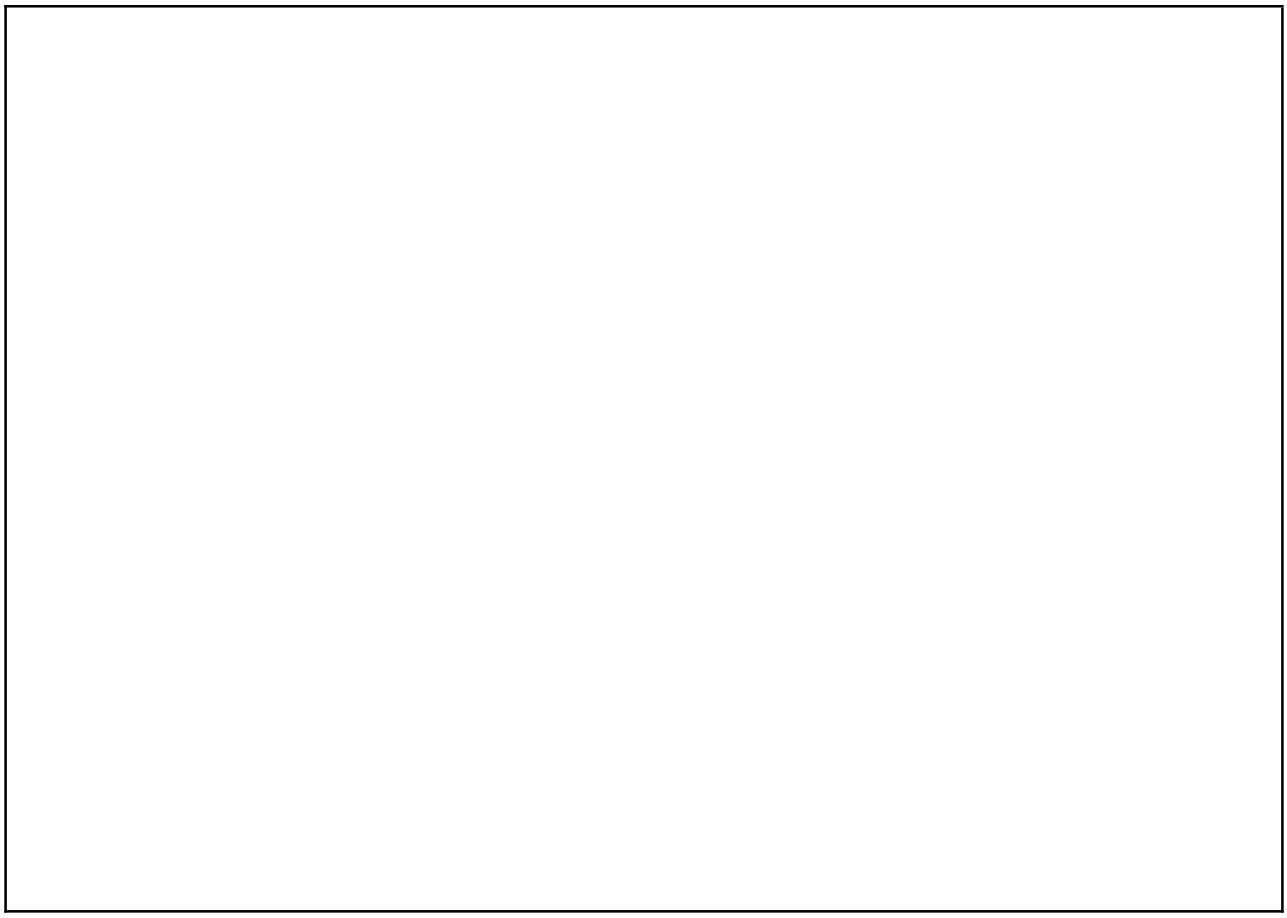
N26	**Control**	82µg/dl	74µg/dl	105µg/dl
N25	**Control**	79µG/Dl	92µg/dl	83µg/dl
N24	**Control**	122µg/dl	85µg/dl	82µg/dl
N23	**Control**	138µg/dl	78µg/dl	73µg/dl
N22	**Control**	121µg/dl	58µg/dl	95µg/dl
N21	**Control**	116µg/dl	68µg/dl	86µg/dl
N20	**Control**	92µg/dl	68µg/dl	77µg/dl
N19	**Control**	87µg/dl	76µg/dl	55µg/dl
N18	**Control**	95µg/dl	54µg/dl	64µg/dl
N17	**Control**	105µg/dl	90µg/dl	52µg/dl
N16	**Control**	113µg/dl	85µg/dl	67µg/dl
N15	**Control**	95µg/dl	93µg/dl	48µg/dl
N14	**Control**	89µg/dl	82µg/dl	75µg/dl
N13	**Control**	123µg/dl	95µg/dl	48µg/dl
N12	**Control**	114µg/dl	108µg/dl	68µg/dl
N11	**Control**	110µg/dl	101µg/dl	77µg/dl
N10	**Control**	103µg/dl	75µg/dl	69µg/dl

N9	**Control**	95µg/dl	84µg/dl	95µg/dl
N8	**Control**	115µg/dl	87µg/dl	82µg/dl
N7	**Control**	98µg/dl	91µg/dl	68µg/dl
N6	**Control**	114µg/dl	73µg/dl	75µg/dl
N5	**Control**	122µg/dl	91µg/dl	93µg/dl
N4	**Control**	128µg/dl	85µg/dl	58µg/dl
N3	**Control**	104µg/dl	76µg/dl	63µg/dl
P1	**Control**	53µg/dl	121µg/d	38µg/dl
P2	**Control**	49µg/dl	136µg/dl	54µg/dl
P3	**Preeclamptic**	67µg/dl	118µg/dl	28µg/dl
P4	**Preeclamptic**	83µg/dl	145µg/dl	42µg/dl
P5	**Preeclamptic**	74µg/dl	112µg/dl	37µg/dl
P6	**Preeclamptic**	69µg/dl	98µg/dl	31µg/dl
P7	**Preeclamptic**	103µg/dl	130µg/dl	24µg/dl
P8	**Preeclamptic**	94µg/dl	84µg/dl	51µg/dl
P9	**Preeclamptic**	86µg/dl	92µg/dl	39µg/dl
P10	**Preeclamptic**	91µg/dl	113µg/dl	24µg/dl

P11	**Preeclamptic**	58µg/dl	78µg/dl	32µg/dl
P12	**Preeclamptic**	62µg/dl	94µg/dl	29µg/dl
P13	**Preeclamptic**	72µg/dl	88µg/dl	42µg/dl
P14	**Preeclamptic**	87µg/dl	103µg/dl	39µg/dl
P15	**Preeclamptic**	113µg/dl	95µg/dl	32µg/dl
P16	**Preeclamptic**	104µg/dl	84µg/dl	42µg/dl
P17	**Preeclamptic**	67µg/dl	104µg/dl	51µg/dl
P18	**Preeclamptic**	82µg/dl	113µg/dl	43µg/dl
P19	**Preeclamptic**	75µg/dl	128µg/dl	28µg/dl
P20	**Preeclamptic**	68µg/dl	119µg/dl	32µg/dl
P21	**Preeclamptic**	55µg/dl	103µg/dl	34µg/dl
P22	**Preeclamptic**	92µg/dl	87µg/dl	40µg/dl
P23	**Preeclamptic**	84µg/dl	76µg/dl	34µg/dl
P24	**Preeclamptic**	63µg/dl	94µg/dl	42µg/dl
P25	**Preeclamptic**	72µg/dl	83µg/dl	18µg/dl
P26		65µg/dl	92µg/dl	26µg/dl

Table 4.2: shows the mean concentrations of selenium (Se), copper (Cu) and zinc (Zn) in pregnant women with or without preeclampsia.

Trace Metal/Parameters	Control (Mean ± SD)	Preeclampsia (Mean ± SD)	F- value	P- value	Cohen's (d) and η^2 (eta squared)
Selenium (µg/dl)	107.79 ± 16.19	76.46 ± 16.58	56.68	< 0.000001	1.92 and 0.48
Copper (µg/dl)	80.58 ± 14.15	103.46 ± 18.70	31.05	0.000001	-1.42 and 0.33
Zinc (µg/dl)	75.84 ± 14.72	35.85± 8.89	153.16	< 0.000001	3.15 and 0.71



From Table 4.2, the mean serum selenium concentration was significantly reduced in the preeclamptic group (76.46 ± 16.58) compared to controls (107.79 ± 16.19). ANOVA confirmed this difference confirmed this difference as highly statistically significant ($F = 56.58$, $p < 0.000001$, $\eta^2 = 0.48$, Cohen's $d = 1.92$) indicating a large effect size and certain physiological differences between the two groups which did not occur randomly.

From Table 4.2, the mean serum copper concentration was greatly increased in the preeclamptic group (103.46 ± 18.70) compared to controls (80.58 ± 14.58). With the statistical analysis used to confirmed the difference as highly statistically significant ($F = 31.05$, $p = 0.000001$, $\eta^2 = 0.33$, Cohen's $d = -1.42$) indicating a moderate to large effect.

From Table 4.2, the mean serum zinc levels were markedly decreased in the preeclamptic group (35.85 ± 8.89) compared to controls (75.84 ± 14.72). The difference was highly significant (F= 153.16, $p < 0.00001$, $\eta^2 = 0.71$, Cohen's d = 3.15). Here the changes in zinc levels induced the largest effect size observed among the three trace metals.

Table 4.3: shows the estimated means of selenium, copper and zinc levels in the control and preeclamptic groups.

Trace Metals / Parameters	Control mean	Preeclampsia mean	p- value	Effect size (η^2)	Cohen's d
Se	108µg/dl	76µg/dl	< 0.000001	0.48	1.92
Cu	81µg/dl	103µg/dl	0.000001	0.33	-1.42
Zn	76µg/dl	36µg/dl	< 0.000001	0.71	3.15

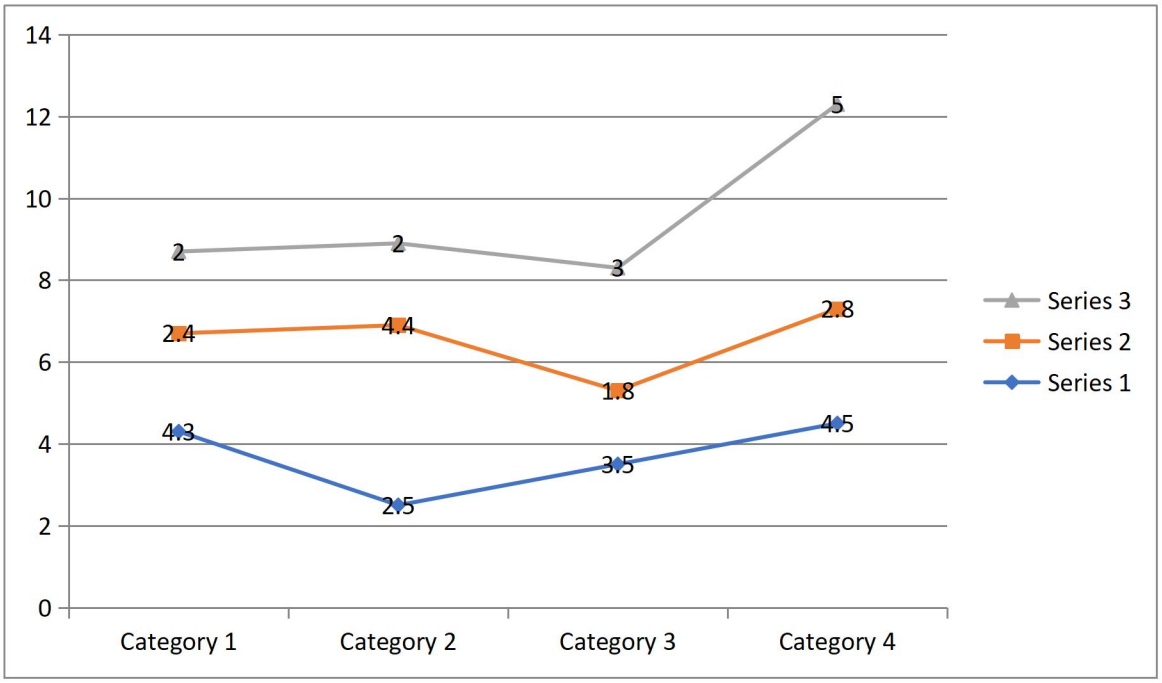


Figure 3.2: Graphical illustration of the mean levels of serum selenium, copper and zinc among pregnant women without preeclampsia

CHAPTER FIVE

DISCUSSION

5.1 Discussion of Findings

This study investigated the relationship between trace metals concentrations (selenium, copper and zinc) in pregnant women with or without preeclampsia. The findings revealed a consistent pattern of reduced selenium and zinc, accompanied by elevated copper levels in preeclamptic women compared to healthy controls. These differences were highly statistically significant with large effect sizes, suggesting that alterations in these trace metals homeostasis play a crucial role in the pathogenesis of preeclampsia.

Selenium was significantly decreased in preeclamptic women. As an essential component of the antioxidant enzyme glutathione peroxidase (GPX), selenium protects endothelial cells against oxidative stress and injury by neutralizing hydrogen peroxide (H_2O_2), lipid peroxide, reactive oxidative species (ROS).

Reduced levels of selenium is involved in the pathogenesis of preeclampsia through impaired antioxidant defense mechanisms which enhances oxidative stress within the placenta with deficiency of selenium also being implicated in endothelial dysfunction and the exaggerated inflammatory response feature of preeclampsia.

In contrast, copper was significantly elevated in the preeclamptic group. While copper is an essential cofactor and component for antioxidant enzymes such as superoxide dismutase (SOD).

Elevated copper levels are involved in the pathogenesis of preeclampsia since it induces Fenton reactions that leads to ROS formation which eventually leads to oxidative stress one of the major

hallmarks of preeclampsia. Thus, elevated maternal copper levels induce pathological effects rather than physiological effects with such pathological effects leading to oxidative stress, lipids peroxidation and vascular injury. In the pathogenesis of preeclampsia, elevated copper levels enhance pro-oxidants- antioxidants imbalance.

Zinc concentrations were markedly reduced in preeclamptic women, showing the strongest effect size among the studied trace metals. Zinc plays a vital role in antioxidant defense since it is a cofactor of the antioxidant enzyme superoxide dismutase (SOD), DNA synthesis, and immune regulation. Its deficiency has been heavily linked to impaired placental development and abnormal trophoblast invasion, one of the central features of preeclampsia.

In the pathogenesis of preeclampsia, reduced levels of zinc are involved in the exacerbation of pro-oxidants and antioxidants imbalance which furthers promote endothelial dysfunction, increased risk and severity of the hypertensive disorder in pregnancy.

The study showed a positive relationship between trace metal imbalance and pathogenesis of preeclampsia observed as combined patterns in the study indicated as low selenium, low zinc and high copper which reveals a profound disruption in micronutrient balance that favours oxidative stress and vascular injury.

This positive relationship supports the oxidative stress hypothesis of preeclampsia which proposes that increased reactive oxidative species generation and reduced antioxidant capacity drive endothelial dysfunction, placental ischemia and other clinical manifestations of preeclampsia.

The significant alterations in trace metals suggest potential diagnostic and therapeutic relevance. Measurement of selenium, copper and zinc could serve as biomarkers for early identification of pregnant women at risk of preeclampsia.

Furthermore, dietary or supplemental correction of selenium and zinc deficiency, along with careful monitoring of copper levels may represent preventive or supportive strategies in the treatment and management of preeclampsia, although more extensive researches still need to be done to establish more preventive or supportive strategies in the management and treatment of this pregnancy induced hypertensive disorder.

In summary, the discussion of this study shows the relationship of the statistical findings to the pathophysiology of preeclampsia thus the clinical importance of this study to maternal health and reproduction.

5.2 Conclusion

This study demonstrated significant alterations in trace metal concentrations among pregnant women with preeclampsia compared to healthy controls. Here, selenium and zinc were markedly reduced, whereas copper levels were significantly elevated in preeclamptic women.

These findings provide strong evidence that trace metal imbalance contributes to oxidative stress, endothelial dysfunction, and placental abnormalities in the pathogenesis of preeclampsia.

The high level of statistical significance ($p \leq 0.000001$) and large effect sizes observed indicate that these differences are not due to chance but represent meaningful biological variations.

Collectively, the results underscore the potential role of selenium, copper and zinc as biomarkers of preeclampsia in its diagnosis and as targets for nutritional and therapeutic interventions in the treatment and management of preeclampsia.

5.3 Recommendations

This study recommends the routine monitoring of trace metals especially selenium, copper and zinc to be considered in antenatal care so as to identify pregnant women at risk of developing preeclampsia. Such monitoring can be done through the estimation of these trace metals using colourimetric, atomic absorption spectrophotometry, inductively coupled plasma mass spectrometry assays. Apart from the use of Se, Zn and Cu as promising biomarkers in the diagnosis of preeclampsia, other promising potential biomarkers also used are calcium, magnesium, uric acid, oxidative stress markers, C- reactive protein, pregnancy associated plasma protein – A among others.

Nutritional supplementation with selenium and zinc, particularly in populations with high prevalence of deficiency may improve antioxidant defense and reduce the risk or severity of preeclampsia. With copper intake carefully balanced to avoid excessive accumulation.

Further longitudinal studies will be required to establish casual relationships between trace metal imbalance and preeclampsia as well as clinical trials being developed to evaluate the effectiveness of targeted micronutrient supplementation in reducing the incidence and complications of preeclampsia.

Awareness programs on maternal nutrition should be done to emphasize the importance of adequate trace element during pregnancy especially among pregnant women attending antenatal clinics. Public maternal health policy such as integration of trace metal screening into maternal health programs may improve early detection of preeclampsia and its prevention strategies

REFERENCES

Dahlen.C.R., Reynolds, L.P.,Caton, J.S, (2022).Selenium supplementation and pregnancy outcomes. *Front.Nutr.*9: 1011850.doi:10.3389/fnut.2022.1011850

Duntas, L.H, (2020). Selenium and at- risk pregnancy: challenges and controversies. Thyroid *Res* 13, 16(2020). https:// doi.org/10.1186/s13044-020-00090-x

Genchi, G., Lauria, G., Catalano, A., Sinicropi, M.S, & Carocci, A. (2023). Biological activity of selenium and its impact on human health. *International Journal of Molecular Sciences* 24 (3) : 2633. https: // doi.org/ 10.3390/ ijms24032633

Goularte, P.D.S, Imperador, C.H.L, Piedade, F.H, Guerin, A.N, Martins, K.D.T, Boreski, D., Gabriel, E.A, Gabriel, S.A, Tiezzi, D.G, Chin, C.M., (2025). Copper, Zinc and Selenium Levels during Pregnancy and Their Relationship with Pre-Eclampsia Risk: A Systematic Review. *Nutrients* 17 (6):1038. https:// doi.org/10.3390/nu17061038

Grieger, J.A,Grzeskowiak, L.E., Wilson, R.L., Bianco-Miotto, T., Perkins, A.V., Norman, R.J., Dekker, G.A. & Roberts, C.T. (2019). Maternal Selenium, Copper and Zinc Concentrations in Early Pregnancy and the Association with Fertility. *Nutrients* 11 (7):1609. https// doi.org/10.3390/nu11071609

Grzeszczak, K., Kwiatkowski, S.& Kosik- Bogacka, D. (2020). The role of Fe, Zn and Cu in Pregnancy. *Biomolecules* 10 (8):1176. https: doi.org/10.3390/biom10081176

Iqbal, S., Ali, I., Rust, P., Kundi, M. & Ekmkcioglu, C. (2020). Selenium, Zinc and Manganese status in Pregnant Women and its Relation to Maternal and Child Complications. *Nutrients* 12(3):725. https:// doi.org/10.3390/nu12030725

Jagodic, J., Pavlovic, S., Borkovic-Mitic, S., Perovic, M., Mikovic, Z., Durdic, S., Manojlovic, D. & Stojsavvljevic , A. (2022). Examination of trace metals and their potential transplacental transfer in pregnancy. *International Journal of Molecular Sciences* 23 (15):8078. https: // doi.org/ 10.3390/ijms23158078

Jin, S., Hu, C. & Zheng, Y. (2022). Maternal Serum Zinc level is associated with risk of preeclampsia: A Systematic review and meta-analysis. *Front Public Health* 10 :968045. https:// doi.org/10.3389/fpubh.2022.968045

Kumari, D., Garg, S. & Bhawrani, P. (2022). Zinc Homeostasis in Immunity and its association with preterm births. *ScandJ Immunol.*95: e13142. https://doi.org/ 10.1111/sji.13142

Minich, W.B. (2022). Selenium metabolism and biosynthesis of selenoproteins in the human body. *Biochemistry (Moscow).*87(Suppl.1): S168- S177. https:// doi.org/10.1134/ S0006297922140139

Narkhede, A.M. & Karnad, D.R. (2021). Preeclampsia and Related Problems. *Indian J Crit Care Med.*25(Supple 3): S261-S266. https://doi.org/10.5005/jp-journals-10071-24032

Nirupama, R., Divyashree, S., Janhavi, P., Muthukumar, S.P & Ravindra, P.V. (2021). Preeclampsia: Pathophysiology and management. *Journal of Gynaecology Obstetrics and Human Reproduction.*50(2): Article 101925. https: // doi.org/10.1016/j.jogoh.2020.101975

Olaoye, T., Oyerinde, O.O, Elebuji, O.J. & Ologun, O. (2019). Knowledge, perception and management of pre-eclampsia among healthcare providers in a maternity hospital. *International Journal of MCH and AIDS* 8(2): 80-88. https:// doi.org/ 10.21106/ijma.275

Otebhi, G.E. & Osadolor, H.B. (2019). Essential Trace Elements Status of Pregnant Women with Pregnancy Complication History in Benin City.

Journal of Applied Sciences and Environmental Management 23(10):1829-1834. https:// doi.org/10.4314/jasem.v2310.10

Oyeyinka, E.K. & Olaniyan, M.F. (2024). Assessment of C-reactive protein, some trace metals and liver enzymes among preeclamptic women in Ibadan. *Journal/Sokoto Journal of Medical Laboratory Science* 9(2):147-159. https: // doi.org/10.4314/sokjmls.v9i2.18

Tanner, M.S., Davey, M.A., Mol, B.W. & Rolnik, D.L. (2022). The evolution of the diagnostic criteria of preeclampsia - eclampsia. *American Journal of Obstetrics and Gynecology* 226(2): S835-S843. https: //doi.org/10.1016/j.ajoy.2021.11.1371

Taysi, S., Tascan, A.S, Ugur, M.G. & Demir, M. (2019). Radicals, oxidative/ nitrosative stress and preeclampsia. *Mini-Reviews in Medicinal Chemistry* 19(3): 178-193. https: // doi.org/ 10.2174/1389557518666181025123511

Turbeville, H.R. & Sasser, J.M. (2020). Preeclampsia beyond pregnancy: Long-term consequences for mother and child. *American Jounal of Physiology- Renal Physiology* 318(6):F1315-F1326. https:// doi.org/ 10.1152/ajprenal.00071.2020

Yin, S., Hongbo, H., Chong, Z., Kai, H., Lirui, H. & Yuhan, F. (2025). The physiological role of copper: Dietary sources, metabolic regulation and safety concerns. *Clinical Nutrition* 48: 161-179. https: //doi.org/ 10.1016/j.clnu.2025.03.023