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GEOPHAGIA, NUTRIENT INTAKES AND POSSIBLE HEALTH OUTCOME OF WOMEN WITH PREGNANCY-INDUCED HYPERTENSION - A CASE-CONTROL STUDY

BY

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DECLARATION

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which to a substantial extent has been accepted for the award of any other degree or diploma at Kwame Nkrumah University of Science and Technology, Kumasi or any other educational institution, except where due acknowledgment is made in the thesis.

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ABSTRACT

Calcium is a major nutrient implicated in pregnancy-induced hypertension (PIH). Aside dietary sources of calcium and other essential nutrients required to prevent adverse pregnancy outcomes, the practice of geophagia (consumption of soil/clay) has been reported to provide calcium needed to prevent hypertension during pregnancy. These soils are shown to also contain significant amount of lead and other heavy metals which have been associated with hypertension. The aim of this study was to determine the relationship between geophagia and hypertensive disorders of pregnancy, and assess the dietary intakes and health outcomes in participants. This study was a case-control involving 30 women with PIH without proteinuria and 70 normotensive pregnant women. Percentage intakes of macronutrients for normotensives were within the Adequate Macronutrient Distribution Range and PIH group recorded higher intakes of carbohydrate (72.75±16.16 %), lower intakes of protein (9.77±5.61 %) and fat (17.15±11.99%). Sources of protein among PIH were mainly from cereal based products. Dietary calcium intakes in both groups were lower than recommended (<1,000 mg/day). Geophagia and energy drink intake was not significantly associated with PIH. Coffee intake significantly increased the risk of hypertension (OR, 4.10; 95% CI 2.10-8.00; p=0.004) while food supplementation during pregnancy significantly reduced the risk of PIH (OR, 0.33; 95% CI 0.17-0.61; p=0.017). Hypertensives recorded impaired fasting blood glucose (5.77±1.71 mmol/L, p=0.051) higher levels of urea (3.60±1.29 mmol/L, p=0.000) and creatinine (382.67±11.17 µmol/L, p=0.000). There was no significant difference in serum calcium and ferritin levels in both groups. Women with PIH practicing geophagia recorded significantly low levels of haemoglobin (8.35±1.91 g/dL, p=0.026), calcium (7.05±1.05 mg/dL, p=0.047) and ferritin (14.89±3.73 ng/dL, p=0.000) than those who did not. The population of pregnant women in this study had considerably low intakes of energy and nutrients. There is the need for measures to ensure adequate maternal nutrition for a positive health and pregnancy outcome.

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LIST OF ABBREVIATIONS

AI	-	Adequate Intakes
ANC	-	Antenatal Clinic
BMI	-	Body Mass Index
BMR	-	Basal Metabolic Rate
BUN	-	Blood Urea Nitrogen
DNA	-	Deoxyribonucleic Acid
EER	-	Estimated Energy Requirement
FAO	-	Food and Agriculture Organization
GH	-	Gestational Hypertension
GSS	-	Ghana Statistical Service
HELLP	-	Haemolysis, Elevated Liver Enzyme, Low Platelet
IUGR	-	Intrauterine Growth Restriction
KATH	-	Komfo Anokye Teaching Hospital
LBW	-	Low Birth Weight
MDA	-	Malondialdehyde
MUAC	-	Mid-Upper Arm Circumference
PE	-	Preeclampsia
PIH	-	Pregnancy-Induced Hypertension
PIHD	-	Pregnancy-induced Hypertensive Disorders
PMTDI	-	Provisional Maximum Tolerable Daily Intake
RDA	-	Recommended Dietary Allowance
RNA	-	Ribonucleic Acid
SGA	-	Small for Gestational Age
SOD	-	Superoxide Dismutase
TAS	-	Total Antioxidant Status
WHO	-	World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Nutrients requirement during pregnancy increases with the stage of pregnancy to meet the nutrient demands for the growth of the foetus as well as to prepare the mother for lactation. The nutrient requirement for the growing foetus, including the formation of muscles, organs, bones, blood, skin and other tissues, is met through the mother's dietary intakes and energy stores. This increased demand on the mother is the reason behind the notion that "a pregnant woman eats for two" (Williamson, 2006).

Apart from the growth of the foetus, the placenta and other maternal tissues such as the breast, uterus and fat stores also undergo various stages of growth with the help of hormones such as the placental lactogen (Picciano, 2003). With this, most pregnant women overestimate their energy and nutrient intakes by increasing portion sizes or adding extra snacks to meet both their needs and that of the foetus (Mahan *et al*., 2007).

Meeting the nutrient needs not only depends on the selection of healthier food but also the ability of the body to increase absorption to minimize nutrient losses. The additional intakes are predominantly beneficial if they are mostly met through the intake of nutrient dense foods and not empty caloric foods. Also, nutrient losses from vomiting, which is common in pregnancy can be controlled by ingesting energy dense foods in smaller portions frequently. This does not only reduce losses, but is also beneficial in controlling nausea and heartburn (Sharlin and Edelstein, 2011). Nutrient dense foods can be provided from whole-grain bread and cereal, legumes, dark green

leafy vegetables, low-fat milk and milk products such as yoghurt, lean meat, fish or poultry, as well as fruits.

Aside food cravings which is common in pregnancy, other cravings during pregnancy include non-food items such as ice, soil, soft stones, etc. which is commonly termed as pica (Myaruhucha, 2009; Johnson, 2017). Ingestion of these non-food substances has been shown to reduce appetite for nutritious foods, which eventually leads to nutritional deficiencies or inadequacies (Crosby, 1982). A common form of pica is the ingestion of soil/ clay, also termed as geophagia. Studies show that these soils contain traces of calcium, iron, zinc, magnesium, potassium, copper and manganese, however, the bioavailability of these minerals cannot be guaranteed and hence its nutritional significance (Hunter and de Kleine, 1984; Ekosse *et al*., 2010; Tayie *et al*., 2013). Geophagic clay has also been reported to inhibit the absorption of nutrients in food, especially iron and zinc, resulting in related deficiencies, including iron deficiency anaemia (Rainville, 1998; Hooda *et al*., 2002; Karimi *et al*., 2002; Tayie, 2004; Williams and Haydel, 2010; Miao *et al*., 2015). Aside the nutritional merit or demerit related to geophagia, these soils may contain heavy metals such as lead, cadmium, arsenic etc. which when introduced into the body, can be detrimental. More importantly, exposure to lead during pregnancy has been associated with the development of pregnancy-induced hypertension (Kennedy *et al*., 2012).

Calcium is a major nutrient associated with hypertension during pregnancies, and the most documented (Roberts *et al*., 2003a; Xu *et al*., 2009). Studies conducted indicate that mothers with low dietary intakes of calcium have increased risk of pregnancy-induced hypertensive disorders (Roberts *et al*., 2003a). Several clinical trials suggest that women receiving supplements of calcium have lower blood pressure, hence the use of calcium supplements as a form of intervention in women diagnosed with

preeclampsia (Duley, 2009; Hofmeyr *et al*., 2014). Other studies indicate that calcium supplementation to prevent preeclampsia or hypertensive disorders of pregnancy is only beneficial among populations with low calcium intakes (Villar and Belizán, 2000). Studies by Eiley and Katz (1998) hypothesized that the ingestion of geophagic clay during pregnancy may provide the calcium needed to improve maternal calcium status as well as reduce the risk of hypertension in pregnancy.

1.2 Problem Statement

Pica practice is common during pregnancy. Most studies conducted indicate that geophagia affects maternal health and outcomes of pregnancy such as low birth weight, preterm delivery, among others (Tayie and Lartey, 1999; Khan and Tisman, 2010; Young, 2010). These negative health outcomes are not only limited to the practice of geophagia, as nutrient intake from food also provides the essentials needed to improve maternal health and improve pregnancy outcomes. Geophagia may result in the excessive exposure to harmful metals such as lead, mercury, arsenic, cadmium etc. which can result in hypertension and dysfunction of the kidney, liver, digestive system, lungs and heart (Kippler *et al*., 2009; Naujokas *et al*., 2013; Selinus *et al*., 2013; Woode and Hackman-Duncan, 2014). Studies conducted on geophagic soils are rather contradicting as some indicate it provides enough iron, calcium, and zinc among others to prevent deficiencies (Hunter, 1993; Tayie *et al*., 2013), whereas other studies report that the presence of kaolin in clay is also able to bind with micronutrients, prevent absorption of these micronutrients and lead to deficiencies (Miao *et al*., 2015). Little is known about how this practice, together with nutrition, affects the health of pregnant women and pregnancy outcomes.

1.3 Objective

The aim of this study was to determine the relationship between geophagia and hypertensive disorders of pregnancy, and assess dietary intakes and health outcome of participants.

1.4 Specific Objectives

The specific objectives are;

1. To assess and compare the nutritional intakes of participants.
2. To investigate the association of geophagia and some practices with hypertension in pregnancy.
3. To determine the effect of high blood pressure on maternal haematology and serum biochemistry.
4. To determine the effect of geophagia on blood pressure, haematology and serum biochemistry.

1.5 Justification

Assessing the nutritional status of pregnant women and some dietary risk factors of pregnancy-induced hypertension will provide health professionals information required to develop interventional strategies pertaining to maternal nutrition, dietary practices and managing pregnant women, especially those with pregnancy-induced hypertensive disorders to prevent adverse pregnancy outcomes.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Hypertension in Pregnancy

Hypertension is a common condition in pregnancy, complicating about 2-10% worldwide (Owiredu, 2008b; Mammaro *et al*., 2009; Osungbade and Ige, 2011; Uzan *et al*., 2011). In Ghana, prevalence of pregnancy-induced hypertension in the rural areas is 0.4% and 3.1% in the urban areas (van Middendorp *et al*., 2013). Hypertension in pregnancy, if not properly managed, can lead to foetal and maternal morbidity, and it is known to be the leading cause of maternal deaths (Uzan *et al*., 2011). According to the National High Blood Pressure Education on Blood Pressure Program (2000), hypertension in pregnancy is classified into 4 main categories. These are namely, gestational hypertension, chronic hypertension, preeclampsia-eclampsia and preeclampsia superimposed on chronic hypertension.

Preeclampsia is the most documented form of hypertension in pregnancy as it is a major cause of maternal and child morbidity and mortality, contributing about 16% worldwide (Ghulmiyyah and Sibai, 2012). The exact cause of preeclampsia is still unclear. The general idea with regards to this condition is that it occurs when there is a problem with the placenta (Redman and Sargent, 2005). Studies conducted suggest that it occurs when the placenta is improperly implanted, genetic and immunological factors, abnormal/exaggerated inflammatory response, and an endothelial disorder with associated vasospasm as well as poor nutrition (Roberts *et al*., 1989; Redman *et al*., 1999; Redman and Sargent, 2005; Srivastava *et al*., 2014). Also, the presence of lead in blood has been implicated in the cases of preeclampsia in Tehran (Vigeh *et al*., 2006).

The risk of preeclampsia increases to twice or five times or 18% in pregnant women with a history of preeclampsia, and 50% in women who develop preeclampsia earlier than 27 weeks of gestation. Also, having a medical history of hypertension, diabetes, gestational diabetes or insulin resistance, kidney disease, thrombophilia, increased serum testosterone, or a family history (first degree relatives) of preeclampsia, obesity, interval between pregnancy (<2yrs or >10yrs), carrying more than one pregnancy, history of abortion and change in sexual partner can increase the risks (Roberts *et al*., 2003b; Barton and Sibai, 2008;Wiznitzer *et al*., 2009). Socio-demographic factors such as age (<18yrs or ≥35yrs), low socioeconomic status and the black race have been identified as risk factors (Owiredu *et al*., 2012; Kintiraki *et al*., 2015; Mol *et al*., 2016). Residence in high altitudes has also been implicated in this disorder because of the limited oxygen supply leading to placental hypoxia (Julian, 2011).

Pregnancy-induced hypertension is a known cause of premature birth, small for gestational age (SGA), intrauterine growth restriction (IUGR), placental abruption and stillbirths, as well as maternal mortality and morbidity (Hauth *et al*., 2000; Kintiraki *et al*., 2015). Short term complication on maternal health includes pulmonary oedema, injury to hepatic cells, oliguria, dysfunction of the nervous system, and haematological complications such as thrombocytopenia (Kintiraki *et al*., 2015).

Pharmacologic and lifestyle modification is essential in the prevention and management of hypertension during pregnancy, which is dependent on gestational age, severity of symptoms and blood pressure. Medications such as Nifedipine have been used over time to improve renal function by decreasing serum urea and creatinine levels and protein in urine (Fenakel *et al*., 1991; Tranquilli *et al*., 1992;

Ismail *et al*., 1993). Complete bed rest and salt restriction is not recommended in hypertension during pregnancy, however, mothers are advised to attain the required pre-pregnancy weight or body mass index (Schneider *et al*., 2011; World Health Organization, 2011). Women with severe preeclampsia receive magnesium sulphate ($MgSO_4$) supplements to prevent eclampsia. The World Health Organization (2011) recommends women at high risk of preeclampsia receive calcium supplements or 75 mg of aspirin.

2.1.1 Gestational Hypertension

This is also known as transient hypertension of pregnancy or chronic hypertension occurring in the latter part of pregnancy i.e. after the 20-week gestation, and usually resolves after delivery. The older term for this form of hypertension is pregnancy-induced hypertension (PIH). About 30% of women with gestational hypertension develop preeclampsia (Salonen Ros *et al*., 2000). Like gestational diabetes, gestational hypertension can lead to hypertension later in life.

2.1.2 Chronic Hypertension

Unlike gestational hypertension, this is a form of hypertension in a pregnant woman with/ without a history of hypertension, occurring before 20weeks gestation with blood pressure exceeding 140/90 mmHg. The prevalence of chronic hypertension is 22% among women of child bearing age, depending on the race, age and body mass index (BMI). It is essential in 90% of the cases and can be secondary to underlying conditions such as diabetes, kidney disease or endocrine disorders, or the use of oral contraceptives etc. About 25% of women with chronic hypertension are likely to develop preeclampsia during their pregnancy (Duley *et al*., 2006; Brown *et al*., 2001).

2.1.3 Preeclampsia-Eclampsia

Preeclampsia, formally known as toxaemia (Chesley *et al*., 1968; Diehl *et al*., 2008), is a group of symptoms which develops in the second trimester of pregnancy. It is the most documented form of hypertension in pregnancy because it poses a great health threat to the mother and foetus. It occurs in 5-10% of pregnancies worldwide, in 10% of first pregnancies, and up to 25% of women with a history of chronic hypertension (National High Blood Pressure Education Program, 2000; Sibai *et al*., 2005; Duley, 2009).

It is characterized by a sudden rise in blood pressure and presence of protein in urine with or without oedema (Pridjian and Puschett, 2002). Preeclampsia can lead to a fatal condition called eclampsia characterized by convulsions, coma or death. The only known cure is delivery of baby. Other symptoms which may or may not occur include rapid weight gain due to a significant increase in body fluids or oedema, abdominal pain, severe headaches, reduced urine output, dizziness, excessive nausea and vomiting (Gupte and Wagh, 2014). Another life-threatening obstetric syndrome which can occur is haemolysis, elevated liver enzymes, low platelet count (HELLP) syndrome. It is considered as a variant of/or complication of preeclampsia (Uzan *et al*., 2011). This involves multiple systems including the blood, lungs, liver, kidney and the central nervous system. The symptoms are mostly mistaken for other conditions such as acute hepatitis, disease of the gall bladder, gastritis, flu, among others. Globally, the mortality rate of the HELLP syndrome is 25%. It is also estimated that 15% of women with preeclampsia will develop this syndrome (Isler *et al*., 1999).

The global prevalence among healthy nulliparous (woman who has never given birth) and primiparous (first time mothers) can range from 3-7% and multiparous (woman

who has given birth several times) is 1-5% depending on the ethnic background (Khan *et al*., 2006; Gong *et al*., 2012). In Ghana, the prevalence is 7.03% (Obed and Aniteye, 2007).

2.1.4 Preeclampsia Superimposed on Chronic Hypertension

This is a term for a woman with chronic hypertension who develops signs or symptoms of preeclampsia after 20weeks of gestation (Brown *et al*., 2001). Prognosis for this condition is poor for both mother and foetus because this results in a much worse sign and symptom than in either preeclampsia or chronic hypertension alone. This condition can be misdiagnosed as worsening chronic hypertension and therefore requires high clinical expertise (National High Blood Pressure Education Program, 2000).

2.2 Overview of Nutrition Requirement during Pregnancy

2.2.1 Energy

The total energy requirement for a pregnant woman is essential to meet the increased requirement for growth, lactation, as well as maintain energy balance for physiological and metabolic processes, muscular activities and the formation of new tissues (Butte and King, 2005). The basal metabolic rate (BMR) which is an essential part of the energy equation measures the energy needed to perform basic functions for life such as the transport of substances in the body, cell metabolism, production of enzymes and hormones, excretion of metabolic products, functioning of vital organs such as the heart, the brain, kidneys, and lungs. The BMR represents between 45-70% of the total energy requirement, depending on the age, gender, body size and body mass composition. The BMR increases with the stage of pregnancy, and is dependent

on the mother's nutritional status before conception and the size of the foetus. If the energy reserves are low, the BMR is also down-regulated (King, 2000).

According to the Institute of Medicine, USA, the Estimated Energy Requirement (EER) is defined as the average dietary energy intake that is predicted to maintain energy balance in healthy normal weight individuals defined by age, gender, weight, height, and level of physical activity, and shown to be consistent with good health (Trumbo *et al*., 2002). This definition indicates that energy requirement during pregnancy may vary depending on the preconception body mass index (BMI) of the individual (Butte and King, 2005). A pregnant woman may therefore add between 150 kcal/day to 500 kcal/day depending on the weight and trimester of pregnancy. These additional calories are needed to meet energy requirement as well as maintain a healthy pregnancy weight (National Research Council, 2010).

2.2.2 Carbohydrate

Carbohydrates provide 4kcalories/gram. Although it is the body's preferred energy source for proper functioning, it is also the fuel for foetal brain. Ample carbohydrate is therefore needed for foetal brain development and to spare protein to be used by the body for growth processes and not for the production of glucose. The Adequate Macronutrient Distribution Range (AMDR) for carbohydrate is 45-65% of total caloric intake, ideally 175 grams/day and not less than 135 grams/day (Whitney *et al*., 2009).

2.2.3 Fibre

Fibre is an indigestible plant material which though does not contribute to the development of the foetus, promotes gastrointestinal health and reduces haemorrhoids and constipation, which is common among pregnant women. The Adequate Intake

(AI) of fibre is 28 g/day. Dietary fibre also helps to control blood sugars and reduce blood pressure (Threapleton *et al*., 2013).

2.2.4 Lipids and Fat

Lipids are essential for the growth of the foetus. They include triglycerides, sterols, phospholipids which are essential for energy production, production of hormones, and transport of fat-soluble vitamins, etc. Fats provide 9 kcal/gram and a source of energy for women at risk of malnutrition. Essential fatty acids, linoleic and linolenic acid (omega-6 or omega-3 respectively) are also necessary for proper eye and brain development of the foetus and in the early years after delivery (Innis and Friesen, 2008). AMDR for dietary fat is 20-35% of total caloric intake (Trumbo *et al*., 2002).

2.2.5 Protein

Adequate protein is needed to provide building blocks for the formation of muscles, collagen, enzymes, and hormones. The body helps to maximize the use of protein by the influence of the growth hormone, which promotes nitrogen retention for protein synthesis (Manson *et al*., 1988). The AI for protein during pregnancy is 71grams/day (National Institute of Health, 2016).

2.2.6 Micronutrients

All nutrients are essential to the development of the foetus as it helps to prevent low birth weight (LBW), birth defects, premature delivery as well as support maternal health. As mineral and vitamin deficiency can occur even in the state of over nutrition, the mother must therefore provide such nutrients through the intake of energy dense foods and reduce intakes of fast food, convenience foods, and soft drinks, among others (Young *et al*., 2004; Cordain *et al*., 2005). Some of these nutrients which are very essential and need to be readily available are those directly involved in blood production and cell growth, specifically, those nutrients involved in

DNA synthesis and those involved in bone development. Some of these nutrients involved in blood production and cell growth include folate, iron, zinc, and vitamin B_{12}. Nutrients involved in bone development are calcium, vitamin D, phosphorus, fluoride and magnesium (Whitney *et al*., 2009).

2.2.6.1 Folate

The requirement for folate increases during pregnancy especially in the early weeks of conception, when mothers are not aware of their condition. It is therefore essential for women who are sexually active to include diets rich in folate or take in folic acid. Folate is essential to ensure the complete closure of the neural tube, which occurs in the early weeks. Deficiency can lead to neural tube defects such as cleft palate, spina bifida .Inadequate dietary folate intake can also affect blood composition and result in anaemia, reduced number of white blood cell or leucopenia and platelet count, thrombocytopenia (Scholl and Johnson, 2000; WHO/FAO Report, 2004). Folate is a methyl group donor which helps in the conversion of homocysteine to methionine to prevent hyperhomocysteinemia, an elevated plasma homocysteine level, which has been implicated in the formation of clots (thrombosis) in veins and arteries. Folic acid acts as an antioxidant to prevent cellular or deoxyribonucleic acid (DNA) damage from free radicals (Joshi *et al*., 2001). Dietary sources include green vegetables, whole grains, fruits and fortified foods. The RDA for folate in pregnancy is 600 µg/day.

2.2.6.2 Vitamin B_{12}

The B vitamins are essential during pregnancy, including B_{12}, which activates the folate cofactors; its deficiency which leads to manifestations of folate deficiency and hyperhomocysteinemia, anaemia and neural disorders (Heart Outcomes Prevention Evaluation, 2006; Moll and Davis, 2017). Vitamin B_{12} is present in animal products

such as fish, meat, eggs and milk products as it is synthesized by algae and bacteria present in foods of animal origin. Complete vegans are required to take in daily doses of the vitamin, or foods fortified with this vitamin (WHO/FAO Report, 2004). The RDA for vitamin B_{12} is 2.6 µg/day.

2.2.6.3 Iron

Due to the increase in blood volume during pregnancy, iron is required to increase the amount of haemoglobin to provide for the needs of the foetus. Having enough stores of iron is also essential as foetal needs of iron take priority over the mother's, creating stores which can last up to the fourth or sixth month after birth. This is evidenced by the report of adequate iron stores among new-borns although their mothers had inadequate stores (Whitney *et al.,* 2009). However, deficiencies in iron can lead to foetal growth restriction, preterm delivery or low birth weight (Scholl, 2005). Most women have inadequate iron stores, especially in developing countries, therefore, are mostly provided with 30-60mg iron supplements a day in their second and third trimesters. Food sources of iron include cereals, fish, poultry, meat and green leafy vegetables (WHO/FAO Report, 2004). Vitamin C present in food is essential in the absorption of iron in plant food as it converts non-haem iron to haem-iron, which is readily absorbed. Sources of iron in foods include green leafy vegetables, beans, lean meat etc. The RDA for iron during pregnancy is 27 mg/day (National Institute of Health, 2016).

2.2.6.4 Zinc

Zinc is essential for protein synthesis and cell development as it is directly involved in DNA and RNA (ribonucleic acid) synthesis. Zinc deficiencies are uncommon as its absorption increases when intakes are low therefore, supplementation of zinc is not advised. Pregnant women taking supplements of more than 300mg of iron per day

need supplements of zinc as excess iron can interfere with the absorption and usage of zinc. Sources of dietary zinc include meat, legumes, and whole grain products, among others (WHO/FAO Report, 2004). The RDA for zinc during pregnancy is 12 mg/day for 18yr olds or less and 11 mg/day for 19-50 year olds.

2.2.6.5 Calcium and Vitamin D

Vitamin D plays an important role in the absorption, metabolism and deposition of calcium. Absorption and retention of calcium increases during pregnancy, especially, in the third trimester when foetal bones begin to calcify. The mother may transfer over 300mg of calcium per day to the foetus. Vitamin D deficiency, therefore, leads to calcium deficiency disorders such as osteomalacia in the mother and rickets in the infant (Need *et al*., 2008). Vitamin D can be synthesized by the body using cholesterol and regular exposure to sunlight. It is also present in milk fortified with vitamin D. Sources of dietary calcium include milk, eggs, yoghurt, etc. The AI for calcium during pregnancy does not increase although calcium supplements are required in women with dietary calcium providing less than 600 mg. Adequate Intake (AI) for calcium during pregnancy is 1,000mg/day. RDA for vitamin D for all ages is 600 IU/day (Ross *et al*., 2011).

2.2.6.6 Antioxidant Micronutrients

Antioxidant micronutrients such as vitamin A, C, E, selenium and zinc can be provided from food sources such as vegetables and fruits. These antioxidants can prevent cell damage by reactive oxygen species which may lead to cardiovascular diseases, diabetes and boost immune response (Bendich, 2001; Taylor, 2001; Agarwal *et al*., 2008). Antioxidants can prevent pro-inflammatory agents present in the diet such as polyunsaturated fatty acid (PUFA) from auto-oxidation. They are also

involved in energy production, neurotransmitters and proteins by being involved in the synthesis of carnitine, catecholamine and collagen (Fang *et al*., 2002).

2.3 Systematic Review on Nutrition in Pregnancy-Induced Hypertensive Disorders

Several studies have been conducted among pregnant women to ensure successful deliveries, and reduce health risks associated with pregnancy and complications which may arise because of their condition. The nutritional assessment of these pregnant women forms a greater part of such researches. These assessments are mostly based on their reported intakes which are normally overestimated or underestimated and may not provide an accurate relationship between their nutritional status and the development of hypertension. This review includes studies reporting maternal dietary intakes, serum levels of micronutrients and supplementation in pregnancy-induced hypertensive disorders.

2.3.1 Search Strategy

The search was based on studies published in Google Scholar, Pubmed, Cochrane library and ScienceDirect databases on the nutritional status of pregnant women with hypertensive disorders. Studies were limited to those conducted between the year 2000 and 2017. The purpose of this review is to provide recent data on the relationship between dietary intakes, micronutrients and hypertension during pregnancy. Keywords entered included hypertension in pregnancy, nutrition in pregnancy, nutrition and hypertension, micronutrients and hypertension, micronutrients and preeclampsia (PE), gestational hypertension (GH), eclampsia (E), chronic hypertension (CE) etc.

2.3.2 Inclusion and Exclusion Criteria

Studies conducted on pregnant women relating nutrition with hypertensive disorders were included. Systematic reviews, animal studies and studies on pregnant women with other complications apart from hypertensive disorders such as renal impairment, HIV, or diabetes were excluded.

2.3.3 Selection Process

The initial search in Pubmed, the Cochrane library, Scholar and ScienceDirect made available over 24,100 references that had potential to be included in the review. A review of references led to the elimination of 15,930 papers which were repeated references. 7,782 papers were rejected as they were either systematic reviews, commentaries, perspectives, opinions or book reviews. The scrutiny of the abstracts of 388 papers led to the elimination of 334 papers as they were either animal studies, hormonal studies or foetal blood pressure. A total of 54 papers were reviewed and grouped into studies assessing pregnancy-induced hypertensive disorders in relation to dietary intakes; serum levels of micronutrients and; supplementation.

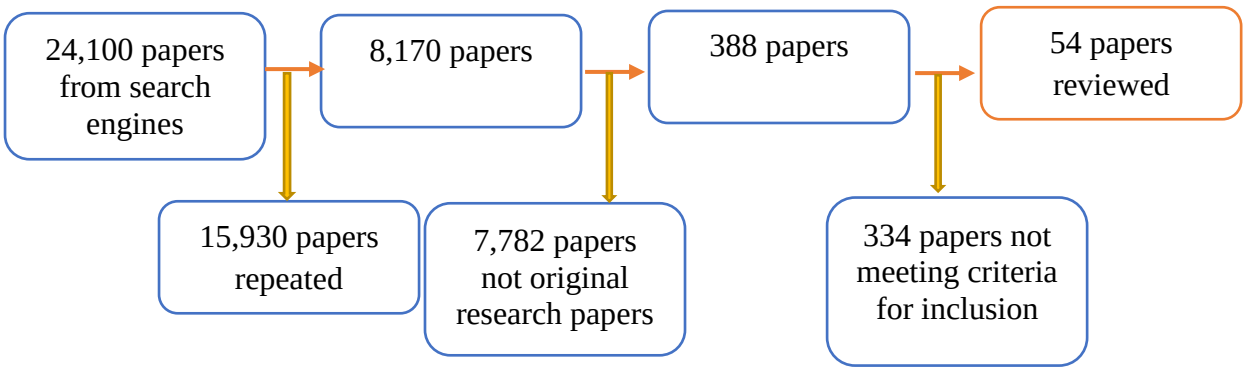

Figure 2.1 Article Selection Process

2.3.4 Data Extraction

Papers were sorted into publication details (Author, date of publication), study site, study design and number of subjects, objectives of studies, dietary assessment tool or supplements, nutrient biomarker, summary of findings.

2.3.5 Results

Maternal Nutrition and Pregnancy-Induced Hypertensive Disorders

Author (s) and year	Study site	Study design/number of subjects	Dietary assessment tool	Nutrient Biomarker	Objective of study	Summary of findings
Hogg *et al*., (2000)	Birmingham, Alabama, USA	Randomised trial / 437 healthy pregnant women.	Supplements of 25mg dose of zinc daily	Homocysteine, haematocrit, plasma folate concentration	To determine whether second-trimester plasma homocysteine levels are elevated among women whose pregnancies are complicated with PIHD	Third trimester homocysteine level higher among women with PIH.
Kharb, (2000)	Rohtak, India	Case control study/ 30 PIHD women and 30 normotensive women	-	Vitamin C and E.	To investigate plasma levels of vitamin C and E in patients with PIHD	Vitamin C and E lower in women with PIHD due to increased free radicals.
Mahomed *et al*., (2000)	Harare, Zimbabwe	Case-control study / 37 eclamptic, 167 preeclamptic and 200 normotensive pregnant women	-	Leukocyte zinc, selenium, and copper concentrations	To assess the risk of PIHD in relation to maternal leukocyte concentrations of selenium, zinc, and copper	Leukocyte selenium and zinc, but not copper, were significantly elevated in PIHD compared with normotensive pregnant women.
Tamura *et al*., (2000)	USA	Double-blind trial / 3,742 pregnant women	Zinc Supplements	Plasma zinc	To evaluate the association between plasma zinc during pregnancy and various measures of pregnancy outcome and neonatal conditions at birth	Plasma zinc concentrations during the late first trimester to the early third trimester not related to PIHD among women of low socioeconomic background.
Adam *et al*., (2001)	Turkey	Case-control study / 20 women with PIHD and 20 control subjects	-	Plasma magnesium, zinc and serum	To determine the change in plasma magnesium, zinc and serum iron concentrations in	Low cellular magnesium levels in women with PIHD. Plasma zinc and serum iron

Author (s) and year	Study site	Study design/number of subjects	Dietary assessment tool	Nutrient Biomarker	Objective of study	Summary of findings
		matched for gestational age		iron concentrations	PIHD	concentrations are unreliable in the management of PIHD.
Morris *et al*., (2001)	USA	Multicentre prospective cohort study of women in a randomized clinical trial/ 4,589 healthy nulliparous women	24-hour dietary recall	-	To determine the effects of nutrient intakes in the development of PIHD among women enrolled in the calcium for Preeclampsia Prevention study	No considerable evidence of nutrition and supplementation associated with hypertensive disorders of pregnancy or pregnancy outcomes.
Hernández-Díaz *et al*., (2002)	North America	Retrospective cohort study / 2,151 mothers of healthy infants	Folic acid or folate containing supplements	-	To investigate the association between folic acid supplementation and PIHD.	Multivitamins containing folic acid may reduce the risk of PIHD.
Ilhan *et al*., (2002)	Elazig, Turkey	Case-control study/ 24 non-pregnant women as controls, 30 normotensive pregnant women and 21 PIHD women in the third trimester	-	Serum zinc, copper levels	To evaluate lipid peroxidation status and antioxidant systems in women with or without PIHD	SOD is consumed by the increased lipid peroxidation in PIHD. This suggests a relationship between increased Cu levels and decreased SOD, Zn levels in pregnancy and preeclampsia
Zhang *et al*., (2002)	Washington, USA	Case-control study / 109 PIHD women and 259 controls	Semi-quantitative food questionnaire	Plasma ascorbic acid	To examine the relationship of reported fruit and vegetable, vitamin C intake and plasma ascorbic levels with PIHD risk.	Increase intake of fruits and vegetables rich in vitamin C and other antioxidants may reduce the risk of PIHD.
Kumru	Elazig,	Case-control study / 30	-	Serum	To investigate differences in	Low levels of maternal serum

Author (s) and year	Study site	Study design/number of subjects	Dietary assessment tool	Nutrient Biomarker	Objective of study	Summary of findings
et al., (2003)	Turkey	women with PIHD and 30 healthy pregnant women.		magnesium, calcium, copper, and zinc	levels of serum magnesium, calcium, copper, and zinc in PIHD and healthy pregnant women.	copper, zinc, and calcium are related to PIHD but may not have a causal role in this disease.
Magri *et al*., (2003)	Malta	Cross-sectional study / 110 normotensive and 33 PIH	-	Serum calcium, magnesium and zinc	To identify any effects that lead, calcium, magnesium, and zinc may have in gestational hypertension.	Blood lead evidently increases blood pressure and development of gestational hypertension.
Williams *et al*., (2003)	Harare, Zimbabwe	Case-control study / 173 women with PIHD and 186 controls	-	Lipid profile, plasma carotenoids, retinol and tocopherol	To examine the relationship between maternal plasma lipoprotein and antioxidant status with risk of preeclampsia	Opposite association between PIHD and HDL cholesterol and antioxidants. PIHD risk with lycopene or γ- and α –tocopherol not clear.
Enquobahrie *et al*., (2004)	Washington, USA	Prospective cohort study / 57 PIHD women as cases, 510 as controls	-	Plasma lipid concentrations	To investigate the relationship between early pregnancy plasma lipid concentrations in the risk of PIHD	Early pregnancy dyslipidemia increases the risk of preeclampsia.
Atamer *et al*., (2005)	Turkey	Cross-sectional prospective study / 32 PIHD, 25 non-pregnant women, 28 healthy pregnant women	-	Serum copper, zinc and selenium	To investigate the changes in enzyme activities of antioxidants and analyse the levels of serum copper, zinc, selenium, leptin and placental MDA and glutathione.	Elevation of lipid peroxides together with impaired antioxidant defence mechanisms and status of trace metals may be related to the pathogenesis of PIHD.
Rytlewski *et al*.,	Krakow, Poland	Prospective, randomized, placebo-controlled study/ 61	3 g of L-arginine	Plasma levels of amino acids	To investigate the influence of dietary supplementation with L-arginine on blood pressure	Continued dietary supplement decreases blood pressure through increased endothelial

Author (s) and year	Study site	Study design/number of subjects	Dietary assessment tool	Nutrient Biomarker	Objective of study	Summary of findings
(2005)		PIHD women			and nitric oxide production in women with PIHD	synthesis and bioavailability of nitric oxide.
Scholl *et al*., (2005)	Camden, New Jersey, USA	Prospective cohort study / 307 pregnant women	24-hour dietary recall	Total antioxidant power in plasma (vitamin A, C, E, B-carotene)	To examine the influence of isoprostane excretion, an indicator of oxidative damage to lipids, and total antioxidant power in the risk of PIHD and its relation to maternal diet during pregnancy	Increased urinary excretion of isoprostane and decreased antioxidant production consistent with oxidative stress, an early indicator of PIHD. The maternal diet influences the production of free radicals.
Olafsdottir *et al*., (2006)	Reykjavik, Iceland	Observational prospective study/ 488 pregnant women	Semi-quantitative food frequency questionnaire	-	To investigate the relationship between maternal intake of cod-liver oil in early and late pregnancy and PIHD.	Consumption of high doses of omega 3 fatty acids in early pregnancy and liquid cod-liver oil may increase the risk of PIHD.
Poston *et al*., (2006)	United Kingdom	Randomized, placebo-controlled trial / 2,410 women at risk of PIHD	Vitamin C (1000 mg daily) and E(RRR α tocopherol, 400 IU) daily	Plasma vitamin C and E and total cholesterol.	To investigate the potential benefit of antioxidants in a cohort of women with a range of clinical risk factors of preeclampsia	Supplements of high dose vitamin C and E does not prevent PIHD in women at risk, but causes low birth weight babies.
Rumbold *et al*., (2006)	Australia	Multicentre, randomized trial / 935 nulliparous women	Food frequency questionnaire / vitamin C, 1000 mg, and E, 400 IU		To assess whether supplementation with vitamin C and E reduced PIHD in nulliparous women.	Daily supplementation does not reduce the risk of PIHD in nulliparous pregnant women
Rumiri	Indonesia	Prospective cohort,	Vitamin and	Superoxide	To evaluate whether, in the	Biochemical and molecular

Author (s) and year	Study site	Study design/number of subjects	Dietary assessment tool	Nutrient Biomarker	Objective of study	Summary of findings
s *et al*., (2006)		randomized, double-blind, placebo-controlled trial/ 299 pregnant women.	mineral supplements	dismutase, Total antioxidant status	selected subjects, antioxidant status could also be used as a first trimester predictive tool for the later development of PIHD	markers of antioxidant status may be clinically useful in the prediction of PIHD.
Serdar *et al*., (2006)	Uludag, Turkey	Case-control study / 30 healthy, 30 mild preeclamptic and 30 severe preeclamptic pregnant women	-	Serum vitamin E, total carotene, cholesterol, copper, iron	To investigate parameters of iron and copper status and oxidative stress and antioxidant function in women with healthy pregnancy, mild and severe preeclampsia	Toxic iron released from ischaemic placental tissue in PIHD increases lipid peroxidation and endothelial cell injury, which is reduced by antioxidant supplementation
Hyppönen *et al*., (2007)	Finland	Prospective cohort study / 2969 women	-	-	To investigate whether risk of PIHD is associated with infant vitamin D supplementation.	Reduced risk of PIHD in the first pregnancy in women, who had received vitamin D supplementation during infancy.
Oken *et al*., (2007)	Massachusetts	Prospective cohort study /1718 pregnant women	Semi-quantitative food frequency questionnaire	-	To examine intakes of calcium, fatty acids, magnesium, folate, vitamins C, D, and E in first trimesters with pregnancy-induced hypertension	Omega-3 fatty acid prevents preeclampsia. No association of calcium, folate, magnesium, n-6 fatty acids and vitamins C, D, and E.
Spinnato *et*	Brazil	Randomized, placebo-controlled, double blind	Supplements of vitamin C (1,000	-	To study whether antioxidant supplementation reduces the	Antioxidant supplementation not effective in reducing the

Author (s) and year	Study site	Study design/number of subjects	Dietary assessment tool	Nutrient Biomarker	Objective of study	Summary of findings
al., (2007)		clinical trial/ 734 women with PIHD	mg) and E (400IU)		incidence of PIHD among patients.	risk of PIHD in women with chronic hypertension.
Owiredu, (2008a)	Ashanti Region, Ghana	Case-control study/ 100 women with PIH and 50 normotensive women	-	Lipid profile and malondialdehyde concentration	To investigate the link between lipidaemia, lipid peroxidation and oxidative stress in women with PIHD.	Women with PIH have higher plasma triglyceride and LDL-cholesterol and lower HDL-cholesterol concentrations
Paknahad *et al*., (2008)	Isfahan, Iran	Case-control study / 46 pregnant women with PIHD and 46 without PIH	Food frequency questionnaire and 24hour dietary recall	-	To determine the nutritional risk factors for PIHD in a group of pregnant women in Isfahan	Lower intake of calcium, zinc, riboflavin and protein possible risk factors for PIH. Adequate intake of dairy products prevents PIH.
Punthumapol and Kittichotpanich, (2008)	Bangkok, Thailand	Cross sectional study / 36 normal, 35 mild PIHD, 33 severe PIHD pregnant women	-	Serum calcium, magnesium and uric acid	To compare serum calcium, magnesium and uric acid in mild, severe preeclamptic women and normal pregnant women.	Hypocalcaemia and hyperuricemia correlated to severe preeclampsia.
Qiu *et al*., (2008)	Washington, USA	Prospective cohort study / 1,538 pregnant women	Food frequency questionnaire	Lipid profile	To assess the relationship between maternal dietary fibre intake, plasma lipids and the risk of PIHD.	High totalfibre intake reduces dyslipidaemia and the risk of PIHD.
Brantsæter *et*	Norway	Prospective cohort study / 23,423	Semi-quantitative food frequency	-	To estimate the association between dietary patterns	High dietary intake of vegetables, plant foods and

Author (s) and year	Study site	Study design/number of subjects	Dietary assessment tool	Nutrient Biomarker	Objective of study	Summary of findings
al., (2009)		nulliparous pregnant women	questionnaire		during pregnancy in the risk of preeclampsia.	vegetable oils decreases the risk of preeclampsia. Consumption of processed meat, sweet drinks, and salty snacks increases the risk.
Catov *et al*., (2009)	Denmark	Prospective cohort study / 28,601 pregnant women	-	-	To relate the frequency and timing of peri-conceptional multivitamin use to the risk of preeclampsia	Folate supplement unrelated to PIHD. Regular multivitamin use associated with a reduced risk of PIHD among normal-weight women.
Klemmensen *et al*., (2009)	Copenhagen, Denmark	Prospective cohort study / 57,346 pregnant women	Food frequency questionnaire	-	To investigate dietary intakes of vitamins C and E and its relationship with the incidence of PIHD.	Low dietary intake of vitamin C increases risk of PIHD or HELLP. Vitamin E from supplements and diet reduces risks.
Kumar *et al*., (2009)	New Delhi, India	Randomized double-blind, placebo-controlled trial / 524 healthy prim gravidas	24-hour dietary recall / 2g of elemental calcium daily	Serum and urinary calcium	To study the effect of calcium supplementation during pregnancy on blood pressure and maternal and neonatal outcomes	Calcium supplementation appears to PIHD in primigravidas who have a daily dietary calcium intake less than the recommended dietary allowances
Akinloye *et al*., (2010)	Osogbo, Osun State, Nigeria	Case-control study / 40 PIHD women, 40 normotensive pregnant women	-	Serum zinc, copper, selenium, manganese and magnesium	To determine the status of zinc, copper, selenium, manganese and magnesium in PIHD pregnant women	Trace elements were significantly reduced in PIHD pregnant women. Dietary supplementation may help prevent PIHD in developing

Author (s) and year	Study site	Study design/number of subjects	Dietary assessment tool	Nutrient Biomarker	Objective of study	Summary of findings
						countries.
Jain *et al*., (2010)	Agra, India	Case-control study/ 50 women with PIHD and 50 normotensive pregnant women	-	Serum calcium, magnesium, and zinc	To analyse and compare concentrations of calcium, magnesium, and zinc in women with PIHD and normal pregnant women	Low calcium, zinc and magnesium may contribute to the risk of PIHD. Supplementation may reduce risks.
Roberts *et al*., (2010)	USA	Multicentre, randomized, double-blind trial / 10,154 women; 5,088 received vitamins, and 5,066 received placebo	Supplements of vitamins C (1000 mg daily) and E(400 IU) daily	-	To assess the effect of antioxidant supplements, vitamins C and E daily, in early pregnancy, in the risk of serious adverse maternal, foetal, and neonatal outcomes related to PIHD among nulliparous women	Supplements of vitamin C and E does not reduce adverse maternal or perinatal outcomes of PIHD among low-risk, nulliparous women
Shand *et al*., (2010)	Vancouver, British Columbia, Canada	Prospective cohort study / 221 women at clinical or biochemical risk for PIHD	-	Serum 25-hydroxyvitamin D (25OHD) concentration	To determine if vitamin D status, based on serum 25-hydroxyvitamin D (25OHD) concentration, was associated with the risk of PIHD	Vitamin D deficiency and insufficiency were common in a group of women at high risk of PIHD, but not associated with subsequent risk of adverse pregnancy outcome.
Ugwuja *et al*., (2010)	Abakaliki, south eastern Nigeria	Case control study / 40 PIHD and 40 normotensive pregnant women	-	Plasma levels of copper, iron and zinc	To compare plasma levels of copper, iron and zinc in PIHD and normotensive pregnant women in a population with high prevalence of deficiencies.	PIHD is associated with significant decrease in plasma copper. .

Author (s) and year	Study site	Study design/number of subjects	Dietary assessment tool	Nutrient Biomarker	Objective of study	Summary of findings
Akhtar *et al*., (2011)	Dhaka, Bangladesh	Cross-sectional study / 60 PIHD women and 30 normotensive women	-	Serum calcium and zinc levels	To observe serum calcium and zinc levels in PIHD women.	Serum calcium and zinc deficiency a risk factor of PIHD. Early supplementation among deficient groups may reduce the incidence of PIHD.
Brantsæter *et al*., (2011)	Norway	Prospective cohort study / 33,399 primiparous women	Food frequency questionnaire	-	To determine the association between consumption of milk-based probiotic products in the development of PIHD.	Regular consumption of milk-based probiotics could be associated with lower risk of preeclampsia in primiparous women
Ringrose *et al*., (2011)	Saskatoon, Saskatchewan, Canada	Case-control study / 78 cases of PIHD, 109 controls	Food frequency questionnaire, 7-day food diary	Serum vitamin D, calcium and albumin.	To examine the association between vitamin D status and hypertension in late pregnancy.	Women with low circulating concentrations of vitamin D are more likely to have hypertension.
Farzin and Sajadi, (2012)	Tehran, Iran	Case-control study / 60 PIHD women and 60 healthy subjects.	-	Serum zinc, selenium, copper, calcium, magnesium	To compare trace elements status in women with or without PIHD.	Levels of Zn, Se, Ca and Mg are significantly altered in pregnant women with PIHD, and these deficiencies not due to haemodilution.
Kim *et al*., (2012)	Seoul, Korea	Case-control study / 30 women with PIHD and 29 normal control subjects	-	Serum calcium, zinc, iron, and selenium	To investigate the relationship between serum mineral levels of zinc, calcium, iron, and selenium and the risk of PIHD in Korean women	Low serum levels of zinc and calcium and high iron levels in PIHD women. These minerals may be associated with the risk of preeclampsia.

Author (s) and year	Study site	Study design/number of subjects	Dietary assessment tool	Nutrient Biomarker	Objective of study	Summary of findings
Ahsan *et al*., (2013)	Dhaka, Bangladesh	Comparative cross-sectional study / 44 preeclampsia, 33 eclampsia, 27 normotensive pregnant women.	-	Serum calcium, magnesium, copper, zinc and iron.	To determine the relation of serum trace elements with preeclampsia and eclampsia.	Significant changes in serum trace element levels were present in preeclampsia and eclampsia which may have a link with its pathogenesis.
Kazemian *et al*., (2013)	Tehran, Iran	Case-control study/113 gestational hypertensives, 150 controls matched	Semi-quantitative food frequency questionnaire	-	To compare energy, macro- and micronutrients intakes of women with gestational hypertension and healthy pregnant women	High intakes of energy, mono and poly unsaturated fatty acids, and low vitamin C, potassium and magnesium increases the risk of PIH. Calcium not associated with risk
Li *et al*., (2013)	Jiangsu and Zhejiang Provinces, China	Prospective cohort study / 215,871 healthy women	-	-	To examine whether maternal supplementation with folic acid only in early pregnancy can prevent PIHD.	Daily consumption of 400μg folic acid alone during early pregnancy cannot prevent the occurrence PIHD.
Sarwar *et al*., (2013)	Bangladesh	Case-control study / 50 PIHD women and 58 normotensives	-	Serum zinc, copper, manganese, and iron	To evaluate the serum levels of four trace elements; zinc, copper, manganese, and iron, in PIHD pregnant women.	Suggests that PIHD patients have considerably low level of serum zinc, copper, manganese, and iron.
Ephraim *et al*.,	Cape-coast, Ghana	Case control study / 120 PIH women, 100 women with PE and	-	Serum Calcium and Magnesium	This study assessed serum Ca^{2+} and Mg^{2+} levels in women with PIH and PE	Serum calcium and magnesium levels are lower in PIH and PE. Mineral supplements may

Author (s) and year	Study site	Study design/number of subjects	Dietary assessment tool	Nutrient Biomarker	Objective of study	Summary of findings
(2014)		160 healthy, age-matched pregnant women.		levels		influence the occurrence of these hypertensive disorders.
Kanagal *et al*., (2014)	India	Double blinded case-control study / 60 PIHD women, 60 normotensive pregnant women	-	Serum calcium and magnesium	To determine the relationship of serum levels of calcium and magnesium in PIHD pregnancies in women from southern coastal India.	Intake of calcium supplements may help in the reduction of incidence of PIHD especially in developing country where nutrition is poor.
Rafeeinia *et al*., (2014)	Gorgan, Iran	Case-control study / 50 healthy pregnant women and 50 women with PIHD.	-	Serum copper and zinc	To assess serum copper, zinc and lipid peroxidation levels in pregnant women with and without PIHD.	Copper, Cu/Zn ratio and malondialdehyde increases blood pressure. May play a role in the risk of preeclampsia
Richards *et al*., (2014)	Cape Town, South Africa	Observational case–control study / 96 PIHD women and 96 controls	Semi-quantitative food frequency questionnaire	Serum calcium and magnesium concentration	To examine the differences in serum concentrations of calcium and magnesium between women with PIHD and normotensive pregnancies	Woman with PIHD showed no difference in calcium status compared to normotensive women. Calcium supplements beneficial in deficient groups to reduce the risk of PIHD.
Amirabi *et al*., (2015)	Iran	Case-control study / 52 PIHD women, 52 normotensive pregnant women	-	Serum levels of calcium, magnesium, copper, and zinc	To determine whether maternal serum levels of calcium, magnesium, copper, and zinc in patients with PIHD are lower than matched control subjects	Calcium, magnesium, copper and zinc between the two groups not significantly different. These trace elements are not involved in the pathogenesis of PIHD.
Vafaei *et al*.,	Kerman, Iran	Case-control study / 40 normotensives, 20 mild	-	Serum levels of calcium,	To evaluate serum levels of calcium, magnesium and zinc	Serum Ca, Mg and Zn levels does not have any clinical

Author (s) and year	Study site	Study design/number of subjects	Dietary assessment tool	Nutrient Biomarker	Objective of study	Summary of findings
(2015)		and 20 severe PIHD pregnancies		magnesium, and zinc.	in PIHD women compared to normotensive ones.	value for predicting and/or managing of PIHD.
Varma *et al*., (2015)	India	Case-control study / 52 women with PIHD, 73 non pregnant, and 65 pregnant normotensive women	-	Serum calcium and magnesium	To compare serum calcium and magnesium levels among PIHD, pregnant and non-pregnant normotensives	Low levels of calcium and magnesium in PIHD pregnant women can be a risk factor
Biswas *et al*., (2017)	Kolkata, India	Case-control study / 50 women with PIH and 50 normotensive primipara	-	Serum copper, iron, zinc, ferritin, calcium and magnesium.	To compare serum levels of copper, zinc, iron, ferritin, calcium and magnesium in normotensive and hypertensive mothers.	Women with hypertension have lower levels of calcium, magnesium, copper, zinc and high levels of ferritin when compared to normotensives.

2.3.6 Discussion

Papers reviewed were those published from the year 2000 to 2017. The studies were either observational or experimental, involving pregnant women with pregnancy-induced hypertensive disorder and/or healthy normotensive pregnant or non-pregnant women.

The first category are studies focusing on maternal dietary intakes of foods to determine the role in developing a form of hypertension in pregnancy. The dietary assessment tool predominantly used was the food frequency questionnaire, followed by the 24-hour dietary recall and one food diary. Frequent intakes of vegetables, fruits, plant foods and plant oils and lower intakes of processed foods, salty foods, and sugary drinks were shown to reduce the risk of developing hypertension in pregnancy (Zhang *et al*., 2002; Williams *et al*., 2003; Brantsæter *et al*., 2009).

Findings indicate that the intake of foods rich in long chain omega-3 fatty acids (docosahexaenoic and eicosapentaenoic) among pregnant women reduces the risk for hypertension in pregnancy, but high doses of liquid cod-liver oils in early pregnancy can have a detrimental effect (Olafsdottir *et al*., 2006; Oken *et al*., 2007). High intake of foods rich in calcium, magnesium, zinc, potassium or folate does not influence the risk of developing hypertension in pregnancy among women in developed countries (Oken *et al*., 2007), but may be beneficial in reducing the risk among women in developing countries or those from low socioeconomic background (Paknahad *et al*., 2008; Kumar *et al*., 2009; Kazemian *et al*., 2013). High energy intakes increase the risk of developing hypertension in pregnancy (Kazemian *et al*., 2013). Low protein intakes also increase the risk of hypertension among women of low socioeconomic background (Paknahad *et al*., 2008). Fibre intake reduces the risk of hypertension by reducing triglyceride concentration and increasing levels of circulating high density

lipoprotein as women with pregnancy-induced hypertension have higher triglyceride and LDL cholesterol concentrations (Williams *et al*., 2003; Enquobahrie *et al*., 2004; Owiredu, 2008a; Qiu *et al*., 2008). Studies by Qui *et al*., (2008) adds that women whose dietary intakes per day contains more than 21g of fibre have a reduced risk of preeclampsia by more than 60% than those with fibre intakes less than 11g per day (RR = 0.33; 95% CI = 0.14–0.79). Also, women whose intakes of total fibre contains more than 13g of soluble fibre had reduced risk of preeclampsia than those with 7.7g of soluble fibre (adjusted RR = 0.35; 95% CI = 0.14-0.87).

Antioxidant dietary intakes such as vitamin E, selenium, and vitamin C consumption within the recommended dietary allowance reduces the risks of hypertension (Zhang *et al*., 2002; Scholl *et al*., 2005), however, high dietary intakes of these micronutrients together with the ingestion of vitamin E supplements may increase the risk of hypertension and lead to low birth weight infants (Poston *et al*., 2006; Klemmensen *et al*., 2009).

Women with low levels of vitamin D intake are likely to develop hypertension in pregnancy (Shand *et al*., 2010; Ringrose *et al*., 2011; Schneuer *et al*., 2014). The daily ingestion of dairy products, particularly, lactobacilli-containing yoghurt, which is rich in calcium and probiotics, has been shown in Norway and Iran to reduce the risk of preeclampsia (Paknahad *et al*., 2008; Brantsæter *et al*., 2011) however, correction of nutritional deficiencies especially, calcium, among women with eclampsia does not affect outcome (Richards *et al*., 2014), indicating that adequate nutrition is effective when used as a preventive tool.

In the second category which focuses on serum micronutrients, calcium, magnesium, zinc and copper levels were found to be higher among women with healthy pregnancy

than women with preeclampsia (Kumru *et al*., 2003; Punthumapol and Kittichotpanich, 2008; Akinloye *et al*., 2010; Jain *et al*., 2010; Akhtar *et al*., 2011; Farzin and Sajadi, 2012, Kim *et al*., 2012; Ephraim *et al*., 2014; Sarwar *et al*., 2013; Kanagal *et al*., 2014; Biswas *et al*., 2017).A decreased calcium intake signals the parathyroid gland to secrete parathyroid hormone. This hormone, together with vitamin D, leads to an increase in intracellular calcium by causing cell membrane permeability. The influx of calcium ions in the intracellular space leads to an increase in vascular smooth muscle contraction, and thus, an elevation in blood pressure. Magnesium plays a role in the relaxation of smooth muscles by acting as a cofactor in peripheral vasodilation. Copper/Zinc Superoxide dismutase (Cu/Zn SOD) acts as an antioxidant which reduces oxidative stress, which is characteristic of preeclampsia (Klotz *et al*., 2003; Beyer *et al*., 2006; Houston and Harper, 2008). Plasma zinc levels have been shown to decrease as pregnancy progresses and therefore not significant in the development of hypertension (Tamura *et al*., 2000, Vafaei *et al*., 2015). A comparative study by Biswas *et al*., (2017) reports that pregnant women with hypertension have elevated serum levels of ferritin when compared to normotensives. Ferritin is basically an iron-storing protein and has been shown to increase during cellular damage or inflammation. This is to prevent unbound iron from becoming destructive, or prevent availability to bacteria to worsen infection among diseased individuals (Kell and Pretorius, 2014). Levels of antioxidant nutrients such as vitamin C, E and selenium are significantly reduced among women with preeclampsia (Kharb, 2000; Atamer *et al*., 2005). Homocysteine levels were shown to be high among women with preeclampsia with reduced levels of folate (Hogg *et al*., 2000;Harma *et al*., 2005;Bergen *et al*., 2012). This signals a role of oxidative stress and the benefit of antioxidant nutrition in preeclampsia (Scholl *et al*., 2005; Serdar *et al*., 2006).

In the supplementation studies, folic acid containing multivitamins were shown to play a role in reducing the risk of hypertension in pregnancy (Hernández-Díaz *et al*., 2002) whereas the ingestion of 400 µg folic acid supplements only does not reduce the risk but may be effective in the prevention of neural tube defects (Catov *et al*., 2009; Li *et al*., 2013).

Studies also show that antioxidant micronutrient supplementation such as vitamin C and E does not prevent the progression of chronic hypertension in pregnancy to preeclampsia or reduce the risk of hypertension in pregnant women (Poston *et al*., 2006; Rumbold *et al*., 2006; Spinnato *et al*., 2007; Roberts *et al*., 2010), therefore routine supplementation of these vitamins during pregnancy is not supported.

Among women with low intakes of dietary calcium, supplementation with calcium is beneficial in reducing the risk of preeclampsia and the rate of preterm deliveries (Kumar *et al*., 2009; Kanagal *et al*., 2014). Women who received supplements of vitamin D during infancy have a reduced risk of developing preeclampsia in their first pregnancy by regulating a long-term immune response (Hyppönen *et al*., 2007). Also, dietary supplementation with the amino acid, L-arginine has been shown to reduce blood pressure among women with preeclampsia by increasing the production of nitric oxide, a vasodilator (Rytlewski *et al*., 2005).

To conclude, among women in the developing countries or of low socioeconomic background, the importance of nutrition is evident in the prevention or reducing pregnancy complications resulting from hypertension. It is therefore necessary for such women to be adequately nourished before conception and during the conception period to ensure a positive pregnancy outcome. Dietary sources of nutrients should be

made available and education on the benefits of such foods promoted, and where necessary, supplementation be given to complement dietary intakes.

2.3.7 Research Gaps

From the above report, it is evident that most of the studies were based in Asia and North America. In Africa, 5 studies have been published, 1 in South Africa, 2 in Nigeria and 2 in Ghana. Most of the studies were concerned with the serum levels of micronutrients, neglecting the influence of dietary habits and intakes on serum levels of the said micronutrients, making public education a challenge as there is limited information on the influence of diet in hypertensive disorders of pregnancy.

2.4 Geophagia

Geophagia is a form of pica used to describe the compulsive and deliberate ingestion of soil, clay or soft stones (Woywodt and Kiss, 2002; Stokes, 2006). Pica is a name given to an abnormal eating behavior in humans, where food and non-food substances or craved for and ingested intentionally in an unusual quantity (Parry-Jones, 1991). This behavior should persist for at least a month before it can be termed as an abnormal behaviour (Rose *et al*., 2000). Terms used to describe the other forms of pica practice include pagophagia, pertaining to the ingestion of ice, freezer frost or iced drinks; amylophagia, ingestion of starch either uncooked starch or dough, or laundry starch; lithophagia, ingestion of soft stones; plasticophagia, ingestion of plastic products; coprophagia, ingestion of faeces; plumbophagia, ingestion of lead, among others (Rose *et al*., 2000; Louw et al., 2007; Uher and Rutter, 2012; Márquez and de Soledad, 2013).

Soils for geophagy are collected from various sources such as from termite mounds, walls; mostly bricks, fields, river beds, mountains, among others. The choice of soil

for geophagy are mainly influenced but not limited to the colour (ranging from white, grey, cream or red in colour), texture of soil, flavour or odour, cleanness of site (Reilly and Henry, 2000; Nchito *et al*., 2004; Tayie, 2004).

Geophagia is the most common form of pica practiced globally (McLoughlin, 1987; Walker *et al*., 1997; Woywodt and Kiss, 2002; Bisi-Johnson *et al*., 2010). Studies conducted in Africa indicate that geophagia and lithophagia are the two most widespread practice among pregnant women (Sule and Madugu, 2000; Tayie, 2004; Ngozi, 2008). In Ghana, geophagia is the most common pica practice among women of reproductive age with a prevalence of 28% and an overall pica prevalence of 48% (Tayie and Lartey, 1999). In Kumasi, pagophagia (abnormal ingestion of ice) is the most common with a prevalence of 41% followed by geophagia with 29.8% and an overall pica prevalence of 47% (Mensah *et al*., 2010).

The type of clay soil commonly ingested in Ghana is the creamy-white clay soil solely mined in a town called Anfoega, in the Volta region of Ghana. It is locally called "shirew" or "ayello" in the local dialect. A freshly mined clay soil is semi-solid in texture due to the water retaining ability of clay soil. The processing procedure for the mined clay involves moulding into lumps and baking in ovens before sale on the market (Tayie *et al*., 2013).

2.4.1 Aetiology of Geophagia

Geophagia is not limited to hunger and poverty but also influenced by race, ethnicity and religion, meaning that cultural beliefs are attached to this practice (Woywodt and Kiss, 2002; Young, 2011). It is a common practice in Africa for a pregnant woman to engage in geophagia (Hunter, 1993). Most cultures in Malawi see it as the most reliable and easiest way to avert nausea, a coping mechanism with the discomforts of

pregnancy and a way of satisfying the unpredictable cravings associated with pregnancy (Diamond, 1998). A woman from such a culture is easily disposed to practice this behaviour during pregnancy. It is also believed to be a means to connect with ancestors, boost fertility and make the skin healthy and beautiful (Woywodt and Kiss, 2002; Njiru *et al*., 2011). In other cultures, food substances are mixed with non-food items as part of its preparation. In America, some cultures such as the Otomac Indians are known to mix acorn dough with geophageous clay before baking (Sing and Sing, 2010).

2.4.2 Possible Health Implications

Geophagia has been associated with a form of adaptation to micronutrient deficiencies such as calcium, zinc, and iron (Singhi *et al*., 2003; Kettaneh *et al*., 2005; Borgna-Pignatti and Zanella, 2016). These micronutrients are essential for growth and development and therefore not a surprise to be a major practice among children and pregnant mothers. Reports on pica practice, specifically geophagia and iron status indicate that pregnant women engaging in this practice have low haemoglobin levels (Tayie and Lartey, 1999). Other studies report that the ingestion of iron and calcium rich soils by people with these deficiencies serves as a form of supplementation (Reid, 1992; Hooda and Henry, 2007). Likewise, pregnant women in Nigeria ingest clay to meet their physiological need for magnesium and calcium (Reilly and Henry, 2000; Abrahams *et al*., 2013,). Some reports indicate that individuals with these identified deficiencies abstained from pica practices after receiving supplements of the respective nutrients, but not true for all studies (Blinder *et al*., 1988; Piazza *et al*., 2002). On another hand, these practices have been known to cause nutrient deficiencies in individuals practicing pica. In an animal study, kaolin, which is a substance present in clay, has been associated with iron deficiency anaemia, as it is

able to bind with iron, and other minerals and prevent its absorption (Hooda *et al*., 2002; Williams and Haydel, 2010; Alexander *et al*., 2015). It is still unclear whether clay ingestion induces iron deficiency or a result of iron deficiency. Although iron deficiencies have strongly been associated with pica practices, only a small number of people with these deficiencies engage in pica practices.

Aside the ability to cause nutritional deficiencies, the ingestion of non-food substances in itself may lead to a loss of appetite for nutritional foods, worsening the deficiency (Crosby, 1982), which is a common habit of people with anorexia nervosa (Woywodt and Kiss, 2002). Apart from iron deficiency anaemia, soil eating is implicated in parasitic infections and developmental problems during pregnancy (Tayie, 2004; Hough, 2007; Tano-Debrah and Bruce-Baiden, 2010; Smit, 2011).

In addition, geophagia in pregnancy is known to be a response to reduced maternal immunity, where the introduction of microbes through geophagy causes the foetus to develop antibodies, thereby building strong immunity against such microbes before birth (Young, 2007; Donovick and Burright, 2013). It is also known to reduce the teratogenic effect of some foods, provide enough calcium for foetal bone formation and prevent hypertension in pregnancy (Eiley and Katz, 1998).

Geophagic clay has been used to reduce the effect of toxins in the body and phytochemicals in plants such as tannins from oak leaves, caffeine in coffee and alkaloids in tubers. This is possible because geophagic clay has a high cation exchange capacity, which is the ability of the soil to hold on to positively charged ions (Johns, 1986; Ghorbani, 2009; Ngole *et al*., 2010). The diatomaceous, kaolin, smectite and fuller's earth also have the ability to detoxify as they are able to bind to toxins released by microbes and also line mucus membranes of the intestine and prevent the

absorption of these toxins, which can also cause intestinal obstruction (Brand *et al*., 2009; Bisi-Johnson *et al*., 2010; Young *et al*., 2010). To prevent the possible dangers from geophagia, smectite and kaolin have been isolated and incorporated into pharmaceutical products to treat gastrointestinal distress and control nausea and vomiting (Young, 2007).

2.4.3 Minerals and Heavy Metals Present in Geophagic Soil in Ghana

In Ghana, women engaged in geophagy consume a daily average of 70g (Tayie *et al*., 2013). Studies conducted in Ghana on the heavy metal compositions of these clay report that it contains high levels of lead, mercury, cadmium, arsenic, cobalt and other heavy metals in amounts higher than the Provisional Maximum Tolerable Daily Intake (PMTDI) (Järup, 2003; Woode and Hackman-Duncan, 2014). Study by Tayie *et al*., (2013) also adds that geophagic clay contains a significant amount of iron and aluminium and the health risk associated with the practice exceeds nutritional benefits.

2.5 Significance of Laboratory Evaluation

2.5.1 Full Blood Count

This test is a common test performed to determine the overall health of an individual and to diagnose conditions of the blood such as anaemia, blood clotting disorder, inflammation, cancer of the blood such as leukaemia etc. It helps in the diagnosis of these conditions by evaluating the composition of the blood which are red blood cells, white blood cells and platelets. An evaluation of red blood cell is mainly to detect anaemia, white blood cells for infections, allergies, inflammation and cancer and platelets for blood clotting disorders. Haemoglobin and haematocrit, and platelet count (thrombocytopenia) can also be used in the diagnosis and detection of the

severity of hypertension in pregnancy. Haemoglobin or haematocrit may be reduced in hypertensive disorders due to haemolysis. Full blood count or complete blood count can be performed in the fasting or fed state (Leeman and Fontaine, 2008).

2.5.2 Kidney Function Test

Blood urea nitrogen (BUN) and creatinine test are the two most common tests performed to assess kidney function. Urea is a product of protein or amino acid metabolism which takes place in the liver. Creatinine is also produced in the muscles from the breakdown of creatine. The kidney is responsible for the excretion of urea and creatinine, therefore, an imbalance in the levels can indicate and monitor renal diseases. Blood urea levels are also monitored in conditions such as hypertension and heart diseases such as myocardial infarction or heart attack, and congestive heart failure. Urea levels can also be affected by excessive protein breakdown, dehydration, among others. Increased serum creatinine levels accompanied with oliguria is also used as an indicator of severe preeclampsia. Protein in urine is also tested to assess kidney function. Fasting is not a requirement for the test for urea or creatinine (Uzan *et al*., 2011; Mustafa *et al*., 2012).

2.5.3 Total Calcium

Calcium in the body is either free, bound to proteins or ionic form. Total calcium normally measures the amount of free calcium circulating in the body. Calcium is involved in many organ functions of the body such as the nerves, heart and bones to aid in transmission of impulses, muscle contraction, bone mineralization, blood clotting, and balance of intracellular water, among others. Due to involvement of calcium in many organ functions, total calcium together with other laboratory tests is used to assess the function of the above organs as well as renal function, intestinal absorption, and the parathyroid gland. The regulation of intracellular calcium has

been shown to play a key role in the aetiology of hypertension. Low levels of serum calcium indicate deficiency in dietary calcium or other nutritional deficiencies such as magnesium, phosphorus, vitamin D. Calcium is also measured to assess the effectiveness of calcium supplementation. Laboratory test for calcium can be performed in the fasting or fed state (Trudeau and Freier, 1967).

2.5.4 Ferritin

Test for ferritin is used together with haemoglobin and haematocrit, and other iron tests such as total iron binding capacity to diagnose anaemia due to iron deficiency and iron overload. It is also an acute phase reactant and can be used to detect inflammation or infections but not for monitoring purposes. Ferritin levels are usually high when there is haemochromatosis, haemolytic anaemia and sideroblastic anaemia, and low in iron deficiency anaemia. Ferritin is an iron-storing protein found in cells normally in the spleen, liver and bone marrow. Damage to these organs can also cause an increase in ferritin levels in the blood. Ferritin test is performed in the fasting state (Kell and Pretorius, 2014).

2.5.5 Fasting Blood Glucose

This test measures the amount of glucose in blood and commonly used in diabetes screening, but also used to detect hyperglycaemia or hypoglycaemia. Fasting blood glucose test is one of the routine tests performed during pregnancy and also for conditions such as hypertension, hypothyroidism, hypoadrenocorticism and hypopituitarism (Tudela *et al*., 2012).

CHAPTER THREE

3.0 SUBJECTS AND STUDY DESIGN

3.1 Study Design and Setting

The case-control study was conducted at the antenatal clinic (ANC) of the Komfo Anokye Teaching Hospital in the Kumasi Metropolis of Ghana. This study design was used to determine the role of geophagia and dietary intakes in the risk of developing hypertension in pregnancy. The study population included pregnant women between the ages 20 and 40 years with singleton pregnancies.

The cases were pregnant women who met the criteria for diagnosing Pregnancy-induced hypertension (PIH) or gestational hypertension stated by the National High Blood Pressure Education Program Working Group (defined as high blood pressure without proteinuria after 20weeks gestation) after assessment by a qualified Obstetrician/Gynaecologist. The control group were normotensive pregnant women without proteinuria in the second or third trimesters.

3.2 Study Area and Health System

The Komfo Anokye Teaching Hospital (KATH) is a major referral hospital and accessible to patients nationwide. It is in the Ashanti regional capital, Kumasi. From the Population and Housing Census in 2010, the total population count of the region was 4,780,380 (Ghana Statistical Service, 2010). The Ghana Demographic and Health Survey in 2014 reported that 98.8% of pregnant women in Ashanti region receive antenatal care from a skilled health provider and 85.6% deliver in a health facility (GSS and Macro, 2014).

3.3 Sample Size

The sample size was determined using Cochrane's formula; $N= Z^2 p (1-p)/d^2$ where N is the sample size, Z is the standard score for the confidence interval of 95% which is 1.96. P is the sample proportion of the prevalence of the combined probability of hypertension complicating pregnancy in Ghana is 1.75% (van Middendorp *et al*., 2013), d is marginal error which is 5%.

$N = z^2 p (1- p) / d^2$

$N= 1.96^2 * 0.0175 (1-0.0175)/0.05^2$

$N= 3.8416 * 0.0175 (0.9825)/0.0025$

$N= 26.42$

The estimated minimum sample size for women with hypertension is therefore, 26.

3.4 Eligibility Criteria (Inclusion and Exclusion Criteria)

Pregnant women with singleton pregnancy between the ages of 20 and 40 years with gestational age of 20 weeks or more were eligible participants for the study. Pregnant women with medical conditions such as diabetes, hypertension, autoimmune disease and kidney diseases or systemic diseases were excluded.

3.5 Sampling Technique and Recruitment Process

A convenience sampling technique was used to select pregnant women attending ANC clinic and those on admission. Pregnant women were interviewed and a total of 100 women who met the criteria for selection were recruited into the study. Interview took place until the allotted sample size was reached. Each participant was given a detailed information about the study and a consent for participation obtained. The participants were grouped according to gestational age (in trimester). The ages were

categorized into three groups, which are; those below 25 years, above 35 years and 25-35yrs of age.

3.6 Data Collection

3.6.1 Questionnaire

A standard interview-based questionnaire was used to obtain data on demography, clinical history, obstetric status, dietary intakes, among others. Subjects completed questionnaires to obtain information regarding maternal age, smoking habits, alcohol intake, nutrient supplements, residence (urban or rural), and family history of hypertension. The list of questions was either open-ended or close-ended for which respondents gave answers to.

3.6.2 Anthropometric Assessment

Anthropometric measurements including pregnancy weight, body mass index, mid upper arm circumference (MUAC) and Body Mass Composition was taken.

In pregnancy, BMI is more appropriate when taken within 10weeks of pregnancy, however, most participants reported for antenatal clinic later or could not recall their pre-pregnancy weight, therefore the actual weight was recorded as well as the Mid Upper-arm Circumference was used. The heights of pregnant women were measured by standing vertical, without footwear on a stadiometer. The Bio impedance analyser (Omron, Japan) was used to calculate the body fat percentage and visceral fat.

3.6.3 Dietary Assessment

Dietary intake was assessed using a qualitative food frequency questionnaire and 24-hour dietary recall. The nutrient intake was determined using the West African Food Composition Table (Stadlmayr *et al.,* 2012). The food frequency questionnaire was used to assess the habitual intakes of participants from the food groups and a two 24-

hour dietary recall was obtained. First recall obtained on the day of interview and the other one obtained via telephone. The days included 1 week day and 1 weekend. This information was obtained randomly from participants without a prior notice. Nutrients estimated from their intakes were grouped as having adequate (≥70% RDA/AI), inadequate (<70% RDA/AI) or excess (> 100% RDA/AI) intakes. Household handy measures were used to estimate portion sizes. The teaspoon, table spoon and dessertspoon were used to estimate quantities such as milk, sugar and oil. The stewing ladle was used to quantify scoops of rice, waakye or stews, and soup ladle for soups and porridges. The matchbox for fish, meat/chicken and the sardine tin for slices of yam or bread.

3.6.4 Blood Pressure Measurement

This was done in accordance with recommendations by the American Heart Association using a digital sphygmomanometer (Omron, Japan). A replicate measurement was taken for each participant and the mean value recorded as the blood pressure. The systolic and diastolic blood pressure was measured.

3.6.5 Urine Sampling

As part of antenatal routine examination, pregnant women visiting the facility reported with 5-10ml of the freshly voided early morning urine in clean, easy access and leak proof containers. Test strips (Combi 10 SGL, Yercon Diagnostic Co. Ltd. China) were dipped into urine samples and removed. After 5-10 seconds, the strips were observed for colour change.

$$\text{Indicator-H}^{+}_{\text{(Yellow)}} + \text{Protein} \rightarrow \text{Indicator}_{\text{(Blue-green)}} + \text{Protein-H}^{+}$$

3.6.6 Biochemical Assessment

Upon recruitment, 7ml of venous blood was collected from each participant by a phlebotomist after an overnight fast (8-12 hours). The blood samples were apportioned into 3 suitable test-tubes for the following determinations: the fluoride tube for fasting blood glucose, the ethylenediaminetetra-acetic acid (EDTA) tube for full blood count and the gel activator tube for kidney function test (urea and creatinine), total calcium and ferritin. Analysis was performed at the Clinical Analysis Laboratory, KNUST.

3.6.6.1 Full blood Count

Using the auto analyser Sysmex XP-300 Haematology Analyzer (North America), White Blood Cells (WBC), Red Blood Cells (RBC), platelets (Plt), haematocrit (Hct) and haemoglobin (Hb) were measured.

Procedure

The blood sample contained in the EDTA tube was mixed uniformly. The mixed sample was then set to the sample probe of the previously booted auto analyser. Pressing the start button aspirates a portion of the blood sample. The result of the analysed sample was printed out by the Sysmex XP-300.

3.6.6.2 Fasting Blood Glucose

The blood sample contained in the sodium fluoride tube preserves glucose in the blood and prevents the blood from clotting. For Fasting blood glucose, blood plasma was used. The FBG was analysed using the spectrophotometer by Randox, RXmonza^{TM}, United Kingdom, which is a semi-automated analyser. The reagent for the quantitative estimation of human plasma glucose was Liquizone Glucose-MR (GOD-POD) produced by Medsource Ozone Biochemicals Pvt. Ltd.

Procedure

The blood sample contained in the fluoride tubes were centrifuged using the Centrifuge 800D, China. The samples were centrifuged at a speed of 3000rpm for 5minutes. A set of sterilized test tubes were prepared and labelled as Standard (S), Blank (B), and Test (T) samples. For the standard (S), 1.0ml of glucose reagent and 10µl of glucose standard (100 mg/dL) was pipetted into tube labelled S. For blank (B), 1.0ml of glucose reagent only was pipetted into tube labelled B. For test (T) samples, 1.0ml of glucose reagent and 10µl of plasma was pipetted into the tube labelled T. This was repeated for each specimen. The contents of the tubes were mixed well and incubated in a water bath at 37˚C for 10minutes. Different shades of the colour pink were observed in Test and Standard tubes only. The contents of each test tube were mixed and transferred into a clean cuvette and inserted into the flow chamber of Randox Xmonsa^{TM} and the absorbance was read against the reagent blank at 505 nm.

3.6.6.3 Urea

Urea was analysed using the Biolabo Diagnostics, Kenza Biochemistry^{TM}, France. Urea was measured using the end-point analysis where the instrument measures the assay absorbance (Reagent+Sample), multiplies it by a factor obtained from the standard and displays the results. Test was performed according to manufacturer's instructions.

Procedure

The blood sample contained in the gel activator tubes was centrifuged using the Centrifuge 800D. The samples are centrifuged at a speed of 3000rpm for 10minutes. This completely separated blood cells from the serum. A set of sterilized test tubes was prepared and labelled as Standard (S), Blank (B), and Test (T) samples. For the

standard (S), 1.0ml of working reagent and 10µl of Urea standard (50 mg/dL) was pipetted into tube labelled S. For blank (B), 1.0ml of working reagent only was pipetted into tube labelled B. For test (T) samples, 1.0ml of working reagent and 10µl of serum was pipetted into the tube labelled T. This was repeated for each specimen. The contents of the tubes were mixed well and incubated in a water bathe at 37˚C for 3minutes. The tubes were retrieved from the water bath and 1.0 ml of alkaline buffer was added to the Standard, Blank, and each of the Test sample. A yellow colour was formed as alkaline buffer was added. The contents of the tubes were mixed well and incubated again in a water bathe at 37˚C for 5minutes. After incubation, a range of colour from yellow to green was observed in Test samples. A green colour was observed in the Standard tube. The Blank tube remained golden yellow (no colour change). The contents of each test tube were transferred into a clean cuvette and inserted into the flow chamber of a warmed (37^{o}C) spectrophotometer manufactured by BiolaboDiagnostics, KenzaBiochemistry^{TM} and the absorbance was read against the reagent blank at 578 nm.

3.6.6.4 Creatinine

Creatinine was analysed with a semi-automated analyser, Biolabo Diagnostics, Kenza Biochemistry^{TM}. Test was performed according to the manufacturer's instructions.

Procedure

The blood sample contained in the gel activator tubes was centrifuged using the Centrifuge 800D. The samples were centrifuged at a speed of 3000rpm for 10minutes to separate blood cells from the serum. A set of sterilized test tubes were prepared and labelled as Standard (S) and Test (T) samples. Equal volumes, 1 ml each, of the working reagent, sodium hydroxide and picric acid was pipetted into the Standard and each of the test samples and mixed. About 100µL of the creatinine standard was

pipetted into the Standard tube and 100µL of each specimen/ serum was pipetted into separate test sample tubes. The resulting concentration was mixed thoroughly and each labelled tube was transferred into clean cuvette to measure the absorbance. The absorbance of standard and test samples was read at 492 nm, 37˚C after 150 sec using the Biolabo Diagnostics, Kenza Biochemistry™.

3.6.6.5 Total Calcium

Serum total calcium was analysed by the Biolabo Diagnostics, Kenza Biochemistry™. The reagent was Liquizone calcium-MR (Arsenazo III Method) manufactured by Medsource Ozone Biomedicals Pvt. Ltd, India.

Procedure

A 1.0ml of Arsenazo III reagent was pipetted into the blank, standard and sample test tube. About 10µL of standard calcium was pipetted into standard test tube and 10µL of sample serum into sample test tube. The contents of the tubes were mixed properly and incubated for 3minutes at room temperature, 27°C. The content of the test tube was transferred into a cuvette and the absorbance was read at 630nm.

3.6.6.6 Serum Ferritin

Ferritin levels were analysed using a semi-automated analyser, Mindray MR-96A, China.

Procedure

A 20µl of standard ferritin solutions (0, 10, 50, 100, 400, and 800 n/L) and serum were dispensed into the coated well plates. About 100µl of the enzyme conjugate reagent was pipetted into each of the wells. The resulting solution was thoroughly mixed for 30 seconds and incubated for an hour at room temperature (27°C). The contents in the wells were disposed of into a waste container by flicking, and the

microtiter wells rinsed 5 times with a washing buffer. Residual water droplets were removed from the wells by striking sharply onto paper towels. About 100 µl of 3, 3', 5, 5'-Tetramethylbenzidine (TMB) substrate, was dispensed into each of the wells and gently mixed for 5seconds, causing a blue colour formation in most wells. The mixture was then incubated in the dark for 20minutes. About 100µl of a stop solution, diluted sulphuric acid, was pipetted into each well and mixed gently for 30seconds, causing a colour change to yellow. The well plates were inserted into the microplate holder of a microtiter reader, Mindray MR-96A, and the absorbance read at 450nm within 15minutes.

3.7 Statistical Analysis

Data was entered and stored in Microsoft Excel and analysed using SPSS version 22.0 (IBM SPSS Inc. Chicago). Categorical variables were analysed using Chi-square and Fischer's test whilst continuous variables were analysed using the unpaired t-test. Data was represented as means and standard deviation. Pearson's correlation was performed to determine the relationship between variables. Risk factors of hypertension were estimated by odds ratio. In all statistical tests, $p < 0.05$ were considered significant.

3.8 Ethical Approval

Approval was sought from the Committee on Human Research, Publication and Ethics of the Kwame Nkrumah University of Science and Technology (CHRPE/AP/002/17), and the Research and Development Office of Komfo Anokye Teaching Hospital (RD/CR16/243). Participation was voluntary and evidenced by a written consent.

CHAPTER FOUR

4.0 RESULTS

4.1 Introduction

This chapter presents the data on the sociodemographic characteristics of participants (cases and controls), dietary intakes, and biochemical data represented in tables.

4.2 Sociodemographic, Clinical and Obstetric Characteristics of Pregnant Women

The total population of respondents were 100 pregnant women, 70 normotensive pregnant women and 30 pregnant women with hypertension. Majority of participants were 25 to 35 years for cases (43.3%) and controls (70%). For blood pressure, 92.9% of the normotensives had systolic readings below 140 mmHg and 90% recorded diastolic lower than 90 mmHg. Among the hypertensive group, however, 70% recorded systolic pressure 140 mmHg or more and for diastolic pressure, 60% recorded 90 mmHg or more. Pregnant women involved in the study mainly belonged to the Akan ethnic group and were urban dwellers within the Kumasi metropolis. Comparing the marital status of pregnant women, 11.4% of the normotensives were single compared to 33.3% of hypertensive group. Over 60% of participants had either completed Junior High School education, Senior High School or tertiary education. More than 50% were employed or traders, however, with cases of hypertension, 33.3% were unemployed, against 17.1% of controls. None of the participants had any chronic condition, however, with family history of hypertension, 28.6% of controls reported a family history of hypertension and 16.7% among the hypertensive group. For parity, 4.3% where nulliparous, 25.7% primiparous and 70% multiparous for controls. Hypertensive group (HG) recorded 13.30%, 23.30%, and 63.30% for

nulliparity, primiparity and multiparity respectively. All participants within the hypertensive group were in the third trimesters of pregnancy with 90% singleton pregnancy. Table 4.1 shows the sociodemographic and obstetric characteristics of respondents.

Table 4.1 Distribution of Sociodemographic, Clinical and Obstetric Characteristics of Respondents

Characteristics	Normotensives (n=70)		Hypertensive (n=30)		p value
	n	%	n	%	
Age					
<25years	5	7.1	6	20	**0.029**
25-35 years	49	70	13	43.3	
>35 years	16	22.9	11	36.7	
Total	70	100	30	100	
Systolic Pressure (mmHg)					
≤12	43	61.5	4	13.3	**0.000**
120-139	22	31.4	5	16.7	
≥140	5	7.1	21	70.0	
Total	70	100	30	100	
Diastolic Pressure(mmHg)					
≤80	54	77.1	5	16.7	**0.000**
80-89	9	12.9	7	23.3	
≥90	7	10.0	18	60.0	
Total	70	100	30	100	
Ethnic Group					
Akan	58	82.9	25	83.3	0.146
Ewe	0	0	1	3.3	
Mole-Dagbani	12	17.1	3	10.0	
Guan	0	0	1	3.3	
Total	70	100	30	100	
Residence					
Urban	66	94.3	29	96.7	0.526
Rural	4	5.7	1	3.3	
Total	70	100	30	100	
Marital Status					
Single	8	11.4	10	33.3	**0.029**
Married	61	87.2	20	66.7	
Separated/Divorced	1	1.4	0	0	
Total	70	100	30	100	
Level of Education					
Primary	18	25.7	2	6.6	0.090
JSS	11	15.7	8	26.7	

Characteristics	Normotensives (n=70)		Hypertensive (n=30)		p value
	n	%	n	%	
SSS	19	27.2	11	36.7	
Tertiary	18	25.7	5	16.7	
None	4	5.7	4	13.3	
Total	70	100	30	100	
Occupation					
Employed	26	37.2	10	33.3	0.057
Trader	32	45.7	8	26.7	
Student	0	0	2	6.7	
Unemployed	12	17.1	10	33.3	
Total	70	100	30	100	
Chronic Condition					
Yes	0	0	0	0	N/A
No	70	100	30	100	
Total	70	100	30	100	
Family History of Hypertension					
Yes	20	28.6	5	16.7	0.157
No	50	71.4	25	83.3	
Total	70	100	30	100	
Parity					
Nulliparous	3	4.3	4	13.3	0.267
Primiparous	18	25.7	7	23.3	
Multiparous	49	70	19	63.3	
Total	70	100	30	100	
Age of gestation					
2nd Trimester	14	20	0	0	**0.004**
3rd Trimester	56	80	30	100	
Total	70	100	30	100	
Type of Pregnancy					
Single	65	92.9	27	90	0.661
No Idea	3	7.2	3	10	
Total	70	100	30	100	

Categorical data is represented as percentages.

4.3 Comparison of Mean Age, Blood Pressure and Anthropometric Measurements of Normotensive and Hypertensive Pregnant women

Systolic blood pressures of normotensive pregnant women were within the normal (115.87±14.75 mmHg) and lower than hypertensive group, with blood pressure within the range for diagnosing hypertension (145.97±22.75 mmHg, p = 0.000). Likewise, diastolic pressure for normotensives was 70.63±11.65 mmHg while that of

hypertensive group was 93.73±14.23 mmHg (p = 0.000). There was no statistically significant difference in the mean age of the hypertensive group (31.67±8.12 years) and that of normotensives (31.61±4.92 years). There was no significant difference between the mid-upper arm circumference (MUAC) and weight measurement among the two groups, however, the hypertensive group recorded higher values than normotensives. Likewise, BMI for the normotensive group was 29.65±4.74 kg/m^2 and that of the hypertensive group 30.44±7.75 kg/m^2. Visceral fat percentage among hypertensive group was lower than in normotensives although not significantly different. Also, body fat percentages among normotensives were higher (41.27±5.82 %) than in hypertensive group (31.49±11.19, p= 0.000). Table 4.2 summarizes the comparison of mean age, blood pressure and anthropometry of normotensives and hypertensive group.

Table 4.2 Comparison of Mean Age, Blood Pressure and Anthropometric Measurements of Normotensive and Hypertensive Pregnant women

Parameter	Normotensives	Hypertensive	P-value
Systolic Pressure(mmHg)	115.87±14.75	145.97±22.75	**0.000**
Diastolic Pressure(mmHg)	70.63±11.65	93.73±14.23	**0.000**
Age (year)	31.61±4.92	31.67±8.12	0.974
MUAC (cm)	31.50±4.11	32.15±6.33	0.606
Weight (kg)	75.26±14.32	76.58±18.57	0.730
Body Mass Index (kg/m^2)	29.65±4.74	30.44±7.75	0.607
Body Fat Percentage (%)	41.26±5.82	31.49±11.19	**0.000**
Visceral Fat (%)	7.01±1.56	5.97±2.70	0.054

MUAC, Mid-Upper Arm Circumference.

4.4 The Dietary Intakes of Normotensive and Hypertensive Pregnant Women

4.4.1 Comparison of Mean Macronutrient and Micronutrient Intake of Pregnant Women

Table 4.3 shows the comparisons of mean nutrient intakes of normotensives and hypertensive group, which is further compared to the Recommended Daily Allowance

(RDA) or Adequate Intakes (AI) of pregnant women. Normotensive pregnant women had adequate intakes of protein and fibre, representing 76.06% and 78.57% of recommended intakes, whereas hypertensive group had inadequate intakes for both protein and fibre, 50.7% and 50% of recommended intakes. Normotensives had excess intakes of carbohydrates (153.14% of adequate intakes) likewise the hypertensive group (127.43% of AI). Although the dietary reference intake (DRI) for total fat is not determined, normotensives consumed 47.11±25.83 g of fat per day compared to 30.61±28.89 g by hypertensives ($p = 0.013$). Both groups had inadequate intakes of micronutrients such as calcium, iron, potassium, vitamin A, vitamin E and folate, however, when the means are compared between the groups, normotensives had higher intakes compared to hypertensive group ($p < 0.001$). Normotensives had adequate intake of magnesium and zinc whereas hypertensive group had inadequate intakes of these micronutrients ($p = 0.001$ and 0.005 respectively). Normotensives and hypertensive group consumed excess sodium, with normotensives consuming 2902.59±1109.41 g (193.53%) and hypertensive group 2094.85±1272.26g (139.67%, $p = 0.005$). Normotensives consumed 126.42% and 125% of phosphorus and selenium respectively, as hypertensive group consumed 95.71% and 88% of phosphorous and selenium respectively. Vitamin C intakes for normotensives was in excess, 101.94±63.82 mg (120%) while hypertensive group had inadequate intakes, 45.16±47.66mg (52.94%, $p = 0.000$).

Table 4.3 Comparisons of Means of Dietary Intakes between Normotensives and Hypertensive Pregnant Women

Dietary Intake	RDA/AI	Normotensive	%RDA/ AI	Interpretation	Hypertensive	%RDA/AI	Interpretation	P-value
Energy (kcal)	N/A	1755.63±795.95	-	-	1261.81±640.42	-	-	**0.002**
Protein (g/d)	71	54.22±22.15	76.06%	Adequate	36.06±27.98	50.70%	Inadequate	**0.003**
Fat (g/d)	ND	47.11±25.83	-	-	30.61±28.89	-	-	**0.013**
Carbohydrate (g/d)	175	267.65±108.32	153.14%	Excess	223.32±106.66	127.43%	Excess	0.063
Fibre (g/d)	28	21.95±10.10	78.57%	Adequate	14.22±9.41	50%	Inadequate	**0.001**
Calcium (mg/d)	**1,000**	309.36±156.40	30.90%	Inadequate	174.98±146.07	17.50%	Inadequate	**0.000**
Iron (mg/d)	**27**	11.79±6.79	44.44%	Inadequate	6.67±4.61	25.93%	Inadequate	**0.000**
Magnesium (mg/d)	**360**	299.10±104.93	83.06%	Adequate	207.14±120.85	57.50%	Inadequate	**0.001**
Phosphorus (mg/d)	**700**	885.28±308.29	126.42%	Excess	670.19±316.42	95.71%	Adequate	**0.003**
Potassium (mg/d)	4,700	2778.04±1432.48	59.11%	Inadequate	1499.90±1285.85	31.91%	Inadequate	**0.000**
Sodium (mg/d)	1,500	2902.59±1109.41	193.53%	Excess	2094.85±1272.26	139.67%	Excess	**0.005**
Zinc (mg/d)	**11**	7.61±3.57	72.72%	Adequate	5.16±3.89	45.45%	Inadequate	**0.005**
Selenium (µg/d)	**60**	75.01±34.39	125%	Excess	53.59±40.34	88%	Adequate	**0.014**
Vitamin A (µg/d)	**770**	218.34±27.91	28.31	Inadequate	72.45±67.46	9.35%	Inadequate	**0.000**
Vitamin C (mg/d)	**85**	101.94±63.82	120%	Excess	45.16±37.66	52.94%	Inadequate	**0.000**
Vitamin E (mg/d)	**15**	6.49±4.34	40%	Inadequate	3.35±1.78	20%	Inadequate	**0.001**
Folate (µg/d)	**600**	338.36±204.32	56.33%	Inadequate	138.40±121.78	23%	Inadequate	**0.000**

Nutrient intakes analyzed from the average of two 24hour dietary recall. Does not include nutrient supplements. Continuous data are presented as means with their standard deviations. RDA represented in bold print, AI in normal print. ND, not determined; N/A, not applicable.

4.4.2 Comparison of Means of Adequate Macronutrient Distribution Range (AMDR) of Normotensive and Hypertensive Pregnant Women.

There were significant differences in the percentage intakes of carbohydrate, protein and fat between normotensive and hypertensive pregnant women (p = 0.003, 0.006, and 0.007 respectively). The intakes among normotensives were within the adequate macronutrient distribution range. Hypertensive group, however, had higher percentage intakes of carbohydrate,(72.75±16.16) and lower percentage intakes of protein and fat (9.77±5.61 and 17.15±11.99 respectively). Table 4.4 shows the comparison of means of adequate macronutrient distribution range of normotensive and hypertensive pregnant women

Table 4.4 Comparison of Means of Adequate Macronutrient Distribution Range (AMDR) of Normotensive and Hypertensive Pregnant Women

Macronutrient	Normotensive	Hypertensive	AMDR	P-value
Carbohydrate %	62.56±9.97	72.75±16.16	45-65	**0.003**
Protein %	13.13±4.50	9.77±5.61	10-35	**0.006**
Fat %	24.30±9.70	17.15±11.99	20-35	**0.007**

4.4.3 Comparison of Sources of Dietary Protein Intakes of Normotensive and Hypertensive Pregnant Women

Normotensive pregnant women had greater consumption of protein from animal sources than hypertensive group (p = 0.024), but had lower intakes of protein from cereals (p = 0.038). Over 45% of sources of protein in hypertensive group were provided from cereal products. There was no significant difference in proteins from legumes and other plants. Protein sources from animal and plant sources were distributed evenly among normotensives than hypertensive group. Table 4.5 summarizes the sources of dietary proteins among pregnant women.

Table 4.5 Comparison of Sources of Dietary Protein Intakes of Normotensive and Hypertensive Pregnant Women

Protein Source	Normotensive	Hypertensive	P-value
Animal Protein (%)	42.76±20.28	29.51±28.15	**0.024**
Cereal Protein (%)	31.30±21.11	45.60±33.80	**0.038**
Legume Protein (%)	10.07±1.42	10.24±1.49	0.185
Other Plants (%)	15.87±12.80	14.65±10.11	0.120

Data presented are sources of dietary protein represented in percentages. Other plants refer to other sources of protein excluding the above mentioned such as vegetables.

4.4.4 Comparison of Frequency of Food Intake Pattern of Normotensive and Hypertensive Pregnant Women

Seventy percent (70%) of hypertensive group consumed whole grain food products daily compared to 41.4% of normotensives (p = 0.008). There was no significant difference in the frequency of intakes of animal proteins in both groups. A greater percentage of normotensives (92.8%) consumed plant protein at least once a week and 66.7% (p=0.010) hypertensive group 66.7% (p =0.010). Among normotensives, 98.6% consumed vegetables at least once a week. Although 53.3% of hypertensives reported that they consumed vegetables daily compared to 35.7% of normotensives, hypertensives reporting monthly intakes were 13.4% against 1.4% among normotensives (p = 0.004). There were no significant differences between the frequency of intakes of fruit and dairy products, however, 26.7% of hypertensives reported to never consume dairy products, compared to 15.7% of normotensives. Table 4.6 represents the patterns of intake from selected food groups.

Table 4.6 Comparison of Frequency of Food Intake Pattern of Normotensive and Hypertensive Pregnant Women

	Distribution of Intakes (%)		
Food Group	**Normotensive**	**Hypertensive**	**p-value**
Whole grain			
Daily	41.40	70.00	**0.008**
Weekly	58.60	30.00	
Animal Protein			
Daily	37.10	36.70	0.575
Weekly	62.90	63.30	
Plant Protein			
Daily	11.40	6.70	**0.010**
Weekly	81.40	60.00	
Monthly	5.70	26.60	
Never	1.50	6.70	
Vegetables			
Daily	35.70	53.30	**0.004**
Weekly	62.90	33.30	
Monthly	1.40	13.40	
Fruits			
Daily	25.70	23.30	0.300
Weekly	70.00	63.30	
Monthly	4.30	10.10	
Never	0	3.30	
Dairy Products			
Daily	10.00	3.30	0.435
Weekly	52.90	53.30	
Monthly	21.40	16.70	
Never	15.70	26.70	

The presented data are the frequency of food intake from the major food groups, represented in percentages.

4.5 Dietary Practices Associated with Hypertension in Pregnancy

Above 90% of respondents did not take in alcohol. Percentages of respondents who took in energy drinks were similar for both cases and controls (33.3% and 27.1% respectively). There were no significant differences between normotensives and hypertensives in the practice of geophagia, alcohol intake and energy drinks. Geophagia (Odds Ratio [OR] 1.09, 95% confidence interval [95% CI]: 0.54-2.21, p =

0.535), and energy drink intake (OR, 1.34; 95% CI: 0.73-2.46, p = 0.346), did not significantly increase the risk of developing hypertension in pregnancy. Alcohol intake was not significantly associated with hypertension in pregnancy (OR, 0.76; 95% CI: 0.27- 2.18, p = 0.550). There was a significant difference between the groups for coffee intake (15.7% of normotensives and 43.3% of hypertensives) and an increased risk for hypertension by 4 times (OR, 4.10; 95% CI: 2.10 – 8.00, p = 0.004). Also, 80% of normotensive pregnant women received supplementation against 56.7% of hypertensives. Supplementation was shown to reduce the risk of hypertension by 67.3% (OR, 0.33; 95% CI: 0.17 – 0.61, p = 0.017). The most common type of supplementation received by both groups was folic acid supplementation. Table 4.7 summarizes the association of dietary practices with hypertension in pregnancy.

Table 4.7 Association of Dietary Practices with Hypertension in Pregnancy

Characteristics	Normotensives (n=70)		Hypertensives (n=30)		P-value	OR (95% CI)
	n	%	n	%		
Geophagia						
Yes	13	18.6	6	20	0.535	1.09 (0.54-2.21)
No	57	81.4	24	80		
Total	70	100	30	100		
Alcohol Intake						
Yes	6	8.6	2	6.7	0.550	0.76 (0.27-2.18)
No	64	91.4	28	93.3		
Total	70	100	30	100		
Energy drinks						
Yes	19	27.10	10	33.3	0.346	1.34 (0.73-2.46)
No	51	72.90	20	66.7		
Total	70	100	30	100		
Coffee						
Yes	11	15.7	13	43.3	**0.004**	4.10 (2.10-8.00)
No	59	84.3	17	56.7		
Total	70	100	30	100		
Supplement						
Yes	56	80	17	56.7	**0.017**	0.33 (0.17-0.61)
No	14	20	13	43.3		
Total	70	100	30	100		
Type of Supplement						
Iron	4	7.1	4	23.5	0.237	N/A
Folic Acid	39	69.6	9	52.9		
Multivitamin	12	21.4	4	23.5		
Zinc	1	1.8	0	0		
Total	56	100	17	100		

Data presented are OR, Odds Ratio, 95% confidence interval. N/A, not applicable.

4.6 Effect of High Blood pressure on Some Haematological and Biochemical Parameters of Pregnant Women

4.6.1 Comparison of Mean Haematological and Biochemical Data of Normotensive and Hypertensive Pregnant Women

The mean fasting blood glucose measurement was within the normal range among normotensives (5.08±1.13 mmol/L) but hypertensives recorded an impaired fasting blood glucose (5.77±1.71 mmol/L) although the differences between the groups were

not statistically significant. Haemoglobin was normal for both groups but slightly higher among normotensives. White Blood Cell was within the normal range for hypertensives and unusually low in normotensives (p = 0.000). Red Blood Cell was within the normal for both groups but slightly lower among normotensives than hypertensives. Haematocrit and mean corpuscular volume were also within the normal range but lower in hypertensives (p= 0.031 and 0.000 respectively). Platelet and lymphocyte count were within the normal range but slightly higher in hypertensives. Neutrophils were significantly higher among hypertensive group, though within the normal range (p = 0.000). For serum parameters, urea and creatinine were within the normal range for normotensives, and significantly higher among hypertensives (p = 0.000). For total calcium and ferritin levels, normal readings were recorded for both groups. Table 4.8 summarizes the comparison of mean biochemical data of normotensives and hypertensives.

Table 4.8 Comparison of Mean Haematological and Biochemical Data of Normotensive and Hypertensive Pregnant Women

Parameter	Normal Range	Normotensives	Hypertensives	P-value
FBG (mmol/L)	3.9-5.6	5.08±1.13	5.77±1.71	0.051
Hb (g/dL)	9.5-15.0	10.49±1.86	10.26±1.95	0.575
WBC (X 10^3/µL)	5.9-16.9	2.90±1.27	10.30±3.17	**0.000**
RBC (10^6/µL)	2.71-4.43	3.87±0.61	3.92±0.71	0.770
Hct (%)	28.0-40.0	36.37±6.21	33.46±5.93	**0.031**
MCV (fL)	81-99	94.16±9.09	85.92±8.37	**0.000**
MCH (pg/cell)	29-32	27.48±2.92	26.21±3.51	0.087
MCHC (g/dL)	31.7-35.7	29.19±1.95	30.61±1.46	**0.000**
Platelet (X10^3/µL)	146-429	206.31±84.09	213.13±75.38	0.690
Lymphocyte (X10^3/µL)	1.0-3.6	1.88±0.96	2.03±0.71	0.415
Neutrophils (X10^3/µL)	3.9-13.1	1.01±0.91	7.41±2.80	**0.000**
Urea (mmol/L)	0.75-2.75	2.65±0.58	3.60±1.29	**0.000**
Creatinine (µmol/L)	35-80	38.76±5.06	382.67±11.17	**0.000**
Calcium (mg/dL)	8.2-9.7	7.57±3.32	7.98±1.41	0.389
Ferritin (ng/mL)	0-116	51.02±48.71	67.73±64.29	0.240

FBG, Fasting Blood Glucose; Hb, Haemoglobin; WBC, White Blood Cell; RBC, Red Blood Cell; Hct, Haematocrit; MCV, Mean Corpuscular Volume; MCH, Mean Corpuscular Haemoglobin; MCHC, Mean Corpuscular Haemoglobin Concentration.

4.6.2 Association between Blood Pressures and Biochemical Data of Pregnant Women

Systolic blood pressure strongly correlated positively with fasting blood glucose, urea, creatinine, white blood cell and neutrophil. A negative correlation was found with mean corpuscular volume, mean corpuscular haemoglobin and platelet, however, the association was not statistically significant. There was a weak positive correlation between systolic pressure and red blood cell, haemoglobin, haematocrit, mean corpuscular haemoglobin concentration, ferritin and total calcium, with p-values > 0.05. No correlation was found between systolic pressure and lymphocyte. Diastolic pressures also correlated significantly with fasting blood glucose, urea, creatinine, white blood cell, haemoglobin, mean corpuscular haemoglobin concentration, and neutrophil. Negative correlations was found with mean corpuscular volume, platelet and total calcium but were not statistically significant. A positive but weak correlation was observed for red blood cell, haematocrit, mean corpuscular haemoglobin, lymphocyte and ferritin. Table 4.9 summarizes the association of blood pressure with biochemical data.

Table 4.9 Association between Diastolic and Systolic Pressures and Biochemical Data of Pregnant Women

	Blood Pressure [r (p-value)]	
Biochemical Parameter	Systolic	Diastolic
Fasting Blood Glucose	**0.204 (0.042)**	**0.252 (0.011)**
Urea	**0.310 (0.002)**	**0.264 (0.008)**
Creatinine	**0.434 (0.000)**	**0.371 (0.000)**
WBC	**0.566 (0.000)**	**0.560 (0.000)**
RBC	0.138 (0.172)	0.178 (0.076)
Haemoglobin	0.120 (0.234)	**0.203 (0.043)**
Haematocrit	0.014 (0.894)	0.072 (0.479)
MCV	-0.193 (0.054)	-0.145 (0.149)
MCH	-0.066 (0.515)	0.011 (0.915)
MCHC	0.177 (0.078)	**0.234 (0.019)**
Platelet	-0.018 (0.857)	-0.084 (0.404)
Neutrophil	**0.593 (0.000)**	**0.581 (0.000)**
Lymphocyte	0.000 (0.999)	0.009 (0.932)
Ferritin	0.048 (0.637)	0.094 (0.350)
Total Calcium	0.020 (0.845)	-0.036 (0.723)

Data presented are the correlation coefficients, r, and p-values of the association between blood pressure and maternal biochemical parameters.

4.7 Effect of Geophagia on Blood Pressure and Biochemical Parameters of Participants

There were significant differences in diastolic pressure, urea, creatinine and WBC between normotensives (NG) and hypertensives practicing geophagia (HG). HG group recorded significantly low levels of haemoglobin (8.35±1.91), haematocrit (28.45±5.38), ferritin (14.89±3.73) and total calcium (7.05±1.05) than hypertensives without geophagia (HN). In all the stated parameters, values recorded for HN were within the normal ranges. Total calcium levels among normotensives without geophagia (NN) were lower than the reference range, but normal among those practicing geophagia within the group. There was no significant difference in systolic and diastolic pressures, fasting blood glucose, urea and creatinine within the normotensive and hypertensive group who practiced geophagia and those who did

not. Table 4.10 shows the effect of geophagia on maternal blood pressure and biochemical parameters

Table 4.10 The effect of Geophagiaon Maternal Blood Pressure and Biochemical Parameters

	Normotensive		Hypertensive		P-value		
Parameter	**None (NN)**	**Geophagia (NG)**	**None (HN)**	**Geophagia (HG)**	**NN+NG**	**HN+HG**	**NG+HG**
Systolic (mmHg)	116.12±15.64	114.77±10.41	146.29±21.4	144.67±29.85	0.706	0.904	0.057
Diastolic (mmHg)	71.68±12.05	66.00±8.62	95.50±14.59	86.67±11.00	0.060	0.132	**0.004**
FBG (mmol/L)	5.00±1.15	5.47±1.01	5.82±1.89	5.53±0.67	0.154	0.544	0.873
Urea (mmol/L)	2.58±0.55	2.95±0.63	3.63±1.27	3.48±1.51	0.072	0.838	**0.000**
Creatinine (µmol/L)	38.64±5.18	38.88±4.67	387.17±12.03	365.01±7.29	0.868	0.577	**0.000**
WBC ($X10^3$/µL)	2.85±1.28	3.10±1.25	10.19±3.41	10.75±2.16	0.526	0.628	**0.000**
RBC ($X10^6$/µL)	3.88±0.63	3.83±0.54	3.97±0.72	3.70±0.67	0.743	0.398	0.684
Hb (g/dL)	10.65±1.87	9.78±1.70	10.73±1.67	8.35±1.91	0.119	**0.026**	0.150
Hct (%)	36.95±6.35	33.85±4.99	34.71±5.47	28.45±5.38	0.068	**0.035**	0.067
MCV (fL)	95.37±8.48	88.88±10.09	88.04±6.67	77.43±9.71	**0.047**	**0.043**	**0.040**
MCH (pg/cell)	27.89±2.58	25.70±3.73	27.08±2.80	22.72±4.13	0.063	**0.048**	0.166
MCHC (g/dL)	29.27±1.92	28.86±2.09	30.95±1.11	29.22±1.97	0.529	0.085	0.728
Plt ($X10^3$/µL)	201.54±84.40	227.23±82.69	198.13±69.82	273.17±71.56	0.327	0.052	0.241
Lym ($X10^3$/µL)	1.84±1.03	2.08±0.57	2.00±0.74	2.12±0.63	0.246	0.716	0.918
Neut ($X10^3$/µL)	1.01±0.87	1.02±1.00	7.34±2.99	7.67±2.62	0.992	0.760	**0.000**
Ferritin (ng/mL)	51.15±46.00	50.44±48.57	80.94±65.56	14.89±3.73	0.978	**0.000**	**0.000**
Calcium (mg/dL)	7.19±2.34	9.26±5.84	8.22±1.41	7.05±1.05	0.233	**0.047**	0.210

FBG, Fasting Blood Glucose; Hb, Haemoglobin; WBC, White Blood Cell; RBC, Red Blood Cell; Hct, Haematocrit; MCV, Mean Corpuscular Volume; MCH, Mean Corpuscular Haemoglobin; MCHC, Mean Corpuscular Haemoglobin Concentration; Plt, Platelet; Lym, Lymphocyte; Neut, Neutrophil

CHAPTER FIVE

5.0 DISCUSSION

Hypertension in pregnancy, if not properly managed, can lead to foetal and maternal morbidity, and it is known to be a leading cause of maternal deaths (Uzan *et al*., 2011). This study assessed maternal dietary intakes and investigated the association of some practices with PIH, and how these are able to influence their general well-being.

In this case-control study, majority of the women with PIH were above 35 years when compared to normotensives. Studies by Ephraim *et al*. (2014) report that advanced maternal age was the most significant risk factor for pregnancy-induced hypertension, with mothers above 40 years at twice as much risk when compared to those aged 25 (OR = 2.14, p = 0.000). In another study by Owiredu *et al*. (2012) on the putative risk factors for pregnancy-induced hypertension, data showed that women aged 24years and below were not at risk of developing hypertension, however, women between 25-29 years were twice as much risk when compared to ≤24 years (aOR 2.2; 95% CI 0.6-7.6) and over 9 times for women aged 35-39 years. A greater percentage of women with PIH had no formal education (13.3%), were single (33.3%) and unemployed (33.3%) when compared to normotensives with 5.7%, 11.4% and 17.1% respectively, suggesting lower socioeconomic status of women with PIH. Studies by Paknahad *et al*. (2008) in Iran also reported a greater percentage (15%) of hypertensive pregnant women having no formal education when compared to the normotensives (3%, $p < 0.05$). In Ghana, studies by Owiredu *et al*. (2012) reported that hypertension in pregnancy is not influenced by maternal education or marital status. All of the pregnant women with PIH were in the third trimester of pregnancy. Similar studies conducted by Owiredu (2008a) and Ephraim *et al*. (2014), all in Ghana on women

with PIH reported that their mean gestational age which was within the third trimester indicating that although PIH occurs after 20 weeks gestation, it is more common in the third trimester. There was no significant difference between the groups for family history of hypertension and parity. Studies have shown that women with family history of hypertension are at an increased risk of developing hypertension in pregnancy (Kazemian *et al*., 2013). Owiredu *et al*. (2012) reports that nulliparity does not increase the risk of hypertension (aOR 1.8; 95% CI 0.8-4.0) but women with a family history of hypertension were 10 times at risk of developing hypertension in pregnancy than those without history (95% CI 1.2-12.2).

Normotensives recorded normal blood pressure compared to women with PIH with pressure above 140/90 mmHg. There was no statistically significant difference in the mean age, BMI, weight, and MUAC between women with PIH and normotensives. Similar studies conducted by Owiredu (2008a) in Ghana reported higher blood pressure readings among PIH women and no significant differences between their ages, however, hypertensive group recorded higher indices of obesity (weight and body mass index). A study by Ephraim *et al*. (2014) also in Ghana also reports higher indices of obesity and high blood pressure among women with PIH with BMI correlating positively with systolic blood pressure ($r=0.575$, $p<0.01$). High body mass index has been associated with hypertension in pregnancy (Fortner *et al*., 2009; Akinloye *et al*., 2010; Ringrose *et al*., 2011) and studies show that obese women are 4 times at risk of hypertension in pregnancy than those within the normal BMI (95% CI 1.3-10.9) (Owiredu *et al*., 2012). MUAC value ≥25.6 cm in pregnancy has been shown to increase the risk of hypertension, (aOR = 2.49, 95% CI = 1.58-3.94) (Endeshaw *et al*., 2014). In women, weight is normally associated with increased body fat percentage, but in this study, women with PIH recorded higher weight and a

significantly low percentage of body fat (normotensive, 41.26±5.82 % and PIH 31.49±11.19 %). The body weight comprises fat mass, fat-free mass and lean body mass. A component of fat-free mass is water which studies by Valensise *et al*. (2004) on the total body water and extracellular water among pregnant women showed that women with gestational hypertension had higher intracellular fluids due to fluid retention hence their increased weight may be due to water retention. Also, the reduced body fat percentage can be explained by their very low energy intakes and therefore reduced store of energy.

Poor nutritional intakes were observed in the overall study population, however, normotensives had higher energy intakes than hypertensives. The low intakes of energy have also been reported in other studies among women with PIH and this has been attributed conceivably as a response to their present condition rather than the cause (Roberts *et al*., 2003a). Dietary fibre intake among normotensives was 21.95±10.10 g/d and 14.22±9.41 g/d for hypertensives. A prospective cohort study by Qiu *et al*. (2008) showed that women who consumed diet containing at least 21.2 g of total fibre per day were at a 67% reduced risk of developing hypertension in pregnancy than those consuming less than 11.9 d/day (RR = 0.33; 95% CI = 0.14-0.79) in addition to a reduction in triglycerides by 11.9 mg/dL and improving HDL-cholesterol. Plasma lipids increase significantly but even more among women with PIH and high intake of total fibre has been shown to have a controlling effect on plasma lipids (Brown *et al*., 1999; Enquobahrie *et al*., 2004; Owiredu, 2008a; Wiznitzer *et al*., 2009). In this study, although hypertensives consumed a greater percentage of carbohydrate than the AMDR (72.75±16.16 %, p = 0.003), they also consumed high amounts of refined sugar (81.99±79.65 g, p-value = 0.055), resulting in the low intakes of fibre (p = 0.001) and micronutrients. Intake of fruits and

vegetables during pregnancy has been shown to significantly reduce the risk of developing PIH (Endeshaw *et al*., 2014).

Low intakes of iron, magnesium and potassium were observed in women with hypertension. Studies show that high intakes of potassium and magnesium reduce the risk of hypertension in pregnancy (Kazemian *et al*., 2013). A prospective cohort study conducted in Denmark on dietary intakes during pregnancy and the risk of hypertension, concluded that calcium, magnesium, folate, vitamins C, D, E or milk, did not affect the risk of hypertension (Oken *et al*., 2007). Another prospective study in USA by Morris *et al*. (2001) also found no association between nutrition and hypertensive disorders of pregnancy.

Dietary intakes of calcium in both groups were lower than the recommended daily allowance. The role of dietary calcium and intracellular calcium has an opposing effect on blood pressure. Dietary calcium regulates serum calcium levels and decreases blood pressure by decreasing vascular smooth muscle tone and resistance of peripheral blood vessels, but when calcium is increased in the intracellular cells, blood pressure increases. This is explained by the role of parathyroid gland which stimulates 1, 25-dihydroxyvitamin D hormone when dietary calcium intake is low. This causes an increase in cell membrane permeability leading to an influx of calcium into cells, thereby increasing intracellular calcium and blood pressure (Ritchie and King, 2000). The role of dietary calcium in blood pressure and the considerably low intakes by hypertensive pregnant women may worsen condition and normotensives may be at risk of developing hypertension later in pregnancy due to their low intakes. Also, because foetal demands for calcium in the third trimester take priority over the mother's, this leads to calcium turnover from bones of the mother. Also, adequate intakes of calcium have been shown to prevent hypertension of foetuses later in life

and improve bone mineralization as babies are fed with calcium-rich breast milk. Recommendations by the World Health Organization (2011) for the prevention and treatment of hypertension in pregnancy, is to administer 1.5-2.0 g of elemental calcium to pregnant women in areas where dietary intakes of calcium are low. The benefits of supplementation with calcium in PIH is however inconclusive and inconsistent. Magnesium intakes on other hand, was also lower than the RDA during pregnancy, but was considerably low among hypertensives (207.14±120.85 mg/d). Magnesium also plays a role in blood pressure regulation by stimulating the release of prostacyclin, a potent vasodilator, from endothelial cells which inhibits the activation of platelets for blood clots. Therefore, low levels of magnesium may predispose an individual to high blood pressure (Jee *et al*., 2002; Kass *et al*., 2012).

Mean dietary antioxidant micronutrient intakes of zinc, selenium, and vitamin C, among normotensive group were adequate but with low intakes of vitamin A and E. The hypertensive group had inadequate intakes of zinc, vitamin A, C, and E with adequate intakes of selenium. Dietary antioxidant micronutrients have been shown to play key role in endothelial function and the prevention of oxidative stress, which has been shown to be involved in PIH (Zhang *et al*., 2002; Scholl *et al*., 2005; Poston *et al*., 2006; Rumbold *et al*., 2006; Klemmensen *et al*., 2009). A randomized placebo-controlled double-blind study in Brazil by Spinnato *et al*. (2007) and in USA by Roberts *et al*. (2010) on the benefits of antioxidant supplementation (1,000 mg vitamin C and 400IU vitamin E) among pregnant women with PIH without proteinuria did not show any beneficial effect. However, among women with low antioxidant intake, supplementation with antioxidant micronutrients is protective against hypertensive disorders (Rumiris *et al*., 2006).

Eighty percent (80%) of normotensives reported to take in supplements compared to 56.7% of hypertensives (p = 0.017). Routine iron supplementation during pregnancy has been shown to have a negative effect on mothers when deficiencies are not present by increasing oxidative stress (Sibai, 2005). From this study, the total study population recorded a poor nutrient intake which is a possible explanation as to why supplementation in this study showed to reduce the risk of hypertension by 67% (aOR 0.33, 95% CI 0.17-0.61). Alcohol intake did not significantly influence the risk of hypertension in pregnancy, which is also reported by Owiredu *et al*. (2012). A greater percentage of hypertensives reported to take in coffee than the normotensives (p = 0.017) and this showed to increase the risk of hypertension by 4 times (aOR 4.1, 95% CI 2.1-8). Another study in Ethiopia showed that coffee intake during pregnancy increased the risk of hypertension, (aOR= 2.16, 95% CI = 1.32-3.53) (Endeshaw *et al*., 2014). Energy drinks and geophagia showed no significant effect in the development of hypertension. A prospective cohort study by Winkelmayer *et al*. (2005) on caffeine consumption in the risk of hypertension among healthy normotensive non-pregnant women showed that the frequency of consumption of caffeinated and decaffeinated coffee did not increase the risk of hypertension, however, consumption of sugared cola, caffeinated or not, increases the risk of hypertension. A review on the benefits of caffeine consumption by Glade (2010) also adds that caffeine consumption in moderate amount is beneficial in healthy non-pregnant adults.

For both hypertensive and normotensive groups, serum calcium levels were lower than the reference ranges (8.2-9.7 mg/dL), with the PIH women having higher levels than normotensives, although not statistically significant. Other studies also showed that calcium levels among hypertensives are higher than normotensives and this

increases with the severity of condition (Magri *et al*., 2003; Ahsan *et al*., 2013; Vafaei *et al*., 2015). A study in Canada by Ringrose *et al*. (2011) reported that calcium levels among hypertensives and normotensives were not significantly different. However, a study by Ephraim *et al*. (2014) in Ghana reported that women with PIH had significantly lower serum levels of calcium (4.99 mg/dL) than normotensives (9.54 mg/dL), with serum calcium correlating negatively with systolic (r= -0.314) and diastolic (r= -0.221) blood pressures but not statistically significant. Studies conducted in India by Varma *et al*. (2015) also indicated that women with PIH had lower levels of serum calcium when compared to their normotensive counterparts (9.34±0.49 mg/dL) and other studies have reported similar findings (Punthumapol and Kittichotpanich, 2008; Baruah *et al*., 2015; Biswas *et al*., 2017). A review by Trumbo *et al*., (2007) reported that the role of calcium in the prevention of PIH was inconsistent and inconclusive.

Serum ferritin levels were higher among hypertensives (67.73±64.29 ng/mL) than normotensives (51.02±48.71 ng/mL) and correlated positively with systolic (r = 0.048) and diastolic (r = 0.094) blood pressure, although did not reach statistical significance. A study by Biswas *et al*. (2017) reported that serum ferritin levels were significantly higher among hypertensives (90.41±37.39 ng/mL) than normotensives (25.71±11.38 ng/mL), with ferritin levels correlating positively with systolic (r = 0.5079, p-value = 0.004) and diastolic (r = 0.0660, p-value = 0.729) blood pressure. Serum ferritin is a reliable indicator for iron stores among healthy individuals, where low concentrations may indicate iron deficiency, however, among unhealthy individuals, increased levels of ferritin signifies cellular damage and in the case of hypertension in pregnancy, haemolysis (Alper *et al*., 2000). High ferritin levels in pregnancy especially in the third trimester has been associated with poor pregnancy

outcomes (Scholl, 2005). The low level of ferritin in healthy pregnancy is explained by the high foetal demand for iron in the third trimester for blood cell production (Lao *et al*., 2000).

A study by Adam *et al*. (2001) showed no significant difference in serum creatinine, haemoglobin, haematocrit and platelet count between hypertensive and normotensive pregnant women although hypertensives recorded higher creatinine levels, and lower haemoglobin, haematocrit and platelet count. He also added that haemoglobin and platelet number are part of parameters to detect haemolysis, and this haemolysis is due to the destruction of red cells by high pressures exerted on them. In another study, haemoglobin levels among pregnant women with hypertension were significantly higher than in normal pregnancy, $p < 0.05$ (Ilhan *et al*., 2002). Haemoglobin levels can increase with increased red cell production because of low oxygen levels, or diseases of the kidney, heart, liver and bone marrow. In hypertension during pregnancy, studies show that the high levels of haemoglobin are due to low plasma volume expansion and not a result of adequate iron status (Yip, 2000). The abnormally elevated levels of urea and creatinine among hypertensives signify impaired kidney function, which when not managed, may lead to chronic kidney disease (Köttgen *et al*., 2009; Hemmelgarn *et al*., 2010). This shows that high blood pressure is harmful to the health and functioning of the kidney. Also, the impaired fasting blood glucose levels among women with PIH may result in gestational diabetes, given blood glucose levels are not monitored and controlled. Owiredu (2008b) reported that women with PIH are at risk of metabolic syndrome.

Iron deficiency anaemia has been widely reported among pregnant women practicing geophagia (Rainville, 1998; Tayie and Lartey, 1999; Tayie, 2004). In this study population, there was no significant effect of geophagia among normotensives,

however, pregnant women practicing geophagia within the hypertensive group had significantly low levels of haemoglobin, haematocrit and ferritin levels. These parameters are indicators of iron deficiency which may complicate maternal disease condition, affect foetal health, lead to low birth weight infants and result in complication during delivery (Kidanto *et al*., 2009; Banhidy *et al*., 2011). Low levels of calcium among the hypertensive group practicing geophagia may result in a further increase in blood pressure and lead to poor prognosis. Studies indicate that geophagic soil contains calcium and other essential nutrients to prevent hypertension in pregnancy, however, the bioavailability of these minerals are debatable as the presence of kaolin in these soils has been shown to also prevent the absorption of these essential nutrients. In this study, geophagia tends to have a beneficial effect on calcium levels among normotensives and detrimental among hypertensives. Although geophagia is common practice during pregnancy, this practice should not be encouraged among pregnant women with health complications.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusion

Pregnant women under study had considerably low intakes of energy and micronutrients which are crucial in the third trimester as foetal demands for nutrients are increased. The specific role of nutrition in hypertension may be inconclusive, but with this study population, adequate nutrition is crucial to prevent adverse pregnancy outcome. Women with PIH have altered haematology parameters, impaired fasting blood glucose and impaired kidney function, hence, have an increased risk of pregnancy and health complications such as anaemia, gestational diabetes and chronic kidney disease. The practice of geophagia during pregnancy may not be associated with pregnancy-induced hypertension, however, this practice is detrimental to maternal health, especially those with pregnancy-induced hypertension.

6.2 Recommendations

A prospective cohort study of maternal dietary intakes in the entire pregnancy period should be conducted as well as blood biomarkers of nutrients to properly determine the role of nutrients in the development of hypertension in pregnancy.

During antenatal visits, pregnant women should be made aware of some dietary practices which are harmful during pregnancy, and increase education regarding the benefit of adequate nutrition.

Nutrient supplementation should be administered to pregnant women, especially those living in low socioeconomic areas to supplement dietary intakes.

6.3 Limitations

Weight or body mass index during the preconception period or before 10weeks gestation were not available as most participants did not recall pre-pregnancy weight or started antenatal visits after 10 weeks. Weighed food record could have been used together with the 24-hour dietary recall to properly estimate dietary intakes. Dietary nutrients analysed did not include mineral or vitamin supplementation.

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APPENDICES

Appendix A

QUESTIONNAIRE

Geophagia and Nutrient intakes of pregnant women

Code; ……..…… Tel.……

Part A: Background – Demographic and Socioeconomic Status

1. Age **……**
2. Ethnic group

   Akan [ ] Ewe [ ] Mole-Dagbane [ ] Guan [ ] Ga-Adangbe [ ]
3. Highest level of formal education

   Primary education [ ] JSS [ ] Secondary education [ ]

   Tertiary education [ ] None [ ]
4. Marital Status

   Married [ ] Single [ ] Separated/divorced [ ] Widowed [ ]
5. Occupation

   Employed [ ] Housewife [ ] Trader [ ] Student [ ]
6. Place of residence

   Urban [ ] Rural [ ]

PART B: Dietary-related Chronic Disease

7. Presence of any chronic condition

Yes [ ]; specify…….. No [ ]

8. Do you have a family history of hypertension or gestational hypertension?

Yes [ ]; specify…….. No [ ]

PART C: Obstetric Factors and Reproductive profile

1. Gestational age; 2nd trimester /13-27wks [ ] 3rd trimester/28-40wks [ ]
2. Parity; Primigravida (1st pregnancy) [ ] Multigravida (≥2nd pregnancy) [ ]
3. Type of pregnancy; Single pregnancy [ ] Multiple pregnancy [ ]

4. Previous history of preeclampsia; Yes [ ] No [ ]

PART D: Pica Practices

1. Have you engaged in any form of pica practice? Yes [ ] No [ ]
2. History of pica practice

   Childhood [ ] Before pregnancy [ ] Previous pregnancy [ ] First time [ ]
3. Did you engage in any pica practice during this pregnancy? Yes [ ] No [ ]
4. Do you still engage in any pica practice? Yes [ ] No [ ]
5. What is/was the most commonly ingested non-food substance (rank if more than 1)

   Soil / Soft stone [ ] Frozen ice cubes [ ] Charcoal [ ]

   Paint chips [ ] Toothpaste [ ] Other; specify……...
6. How often is/was it ingested?

   6 or more/day [ ] 4-5/ day [ ] 2-3/day [ ] 1/day [ ]

   5-6/week [ ] 2-4/week [ ] 1/week [ ]
7. Reason for consumption of non-food items (rank if more than 1)

   Unknown cravings [ ] Pleasant taste, smell and texture [ ]

   Influence from other pregnant women [ ]

   Normal habit during pregnancy [ ] Idleness [ ] Thirst [ ]

PART E: Dietary pattern

How often do you consume foods from the under listed food groups

Food Group	**Frequency**				
	≥1/day	3-4 /wk	1/wk	Monthly	Never
Whole grain (corn, rice, millet, wheat etc.**)**					
Animal protein (meat, poultry, fish, eggs etc.**)**					
Plant protein (soybeans, beans, groundnuts etc.**)**					
Vegetables (Cabbage, lettuce, tomato, onions, carrots etc.**)**					
Fruits (banana, pawpaw, orange, apple etc.**)**					
Dairy products (milk, yoghurt, cheese etc.**)**					

24-hour Dietary recall

Time	Meal	Quantity

Are you a vegetarian? Yes [ ] No [ ]

Do you take in any form of vitamin / mineral supplement? Yes [ ] No [ ]

If yes, specify; …….

Do you smoke? Yes [ ] No [ ]

Do you take in alcohol? Yes [ ] No [ ]

Do you take in coffee? Yes [ ] No [ ]

Do you take in energy drinks? Yes [ ] No [ ]

PART F: Physical Measurements

MUAC……Ht……Wt…….

Ooedema…… Blood pressure……

Body Mass Composition …….

…….

……..

Urinalysis; Protein……pH……

Glucose……..etc…….....…….....

……

PART G: Blood Parameters

Fasting blood glucose…….

Appendix B

CONSENT FORM

Participant Information Leaflet and Consent Form

This leaflet must be given to all prospective participants to enable them know enough

about the research before deciding to or not to participate

Title of Research: Geophagia and other pica practices; a risk factor for preeclampsia in pregnant women.

Name(s) and affiliation(s) of researcher(s): This study is being conducted by Dr. Christopher Larbie and Miss. Deborah SakuaSackey of the Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

Background: Pica is used to describe an eating disorder in humans where there are cravings for non-food or non-nutritive substances and are ingested in an unusual quantity, persisting for at least a month. This form of eating disorder is widely known among pregnant women. Geophagia, the consumption of clay, soil or chalk, has been linked to hyperkalemia, intestinal blockage, lead poisoning and phosphorus intoxication, microbial infection and iron deficiency anaemia. Other pica practices include ingestion of soft stones, paint chips, laundry starch, and charcoal, among others. Preeclampsia is a group of symptoms which develops in the second trimester of pregnancy, characterized by high blood pressure, proteinuria and/or ooedema. The exact cause of preeclampsia is still unclear, however, improper implantation of the placenta, genetic and immunological factors, and abnormal inflammatory response has been associated with the development of preeclampsia

Purpose(s) of research: The aim of this study is to determine how geophagia and other pica practices increase the risk of developing preeclampsia in pregnant women.

Procedure of the research, what shall be required of each participant and approximate total number of participants that would be involved in the research:
A total of 100 participants will be randomly selected. Information concerning pica practices, socio-demographic characteristics and reproductive profile of each participant will be obtained. Participants will undergo a dietary assessment and blood pressure measurement. Urine samples will be obtained and tested for proteins. Blood samples will be tested for fasting blood glucose level, urea, creatinine, haemoglobin, calcium and ferritin.

Risk(s): You may experience pain when blood sample is being drawn.

Benefit(s): The goal of this research is to determine the relationship between pica practices and preeclampsia. This research will seek to educate expectant mothers involved in the study to increase control over their health to aid in safe deliveries and healthy babies.

Confidentiality: All information collected in this study will be given code numbers.

Voluntariness: Participating in this study should be out of your own free will. You are not under obligation to. Research is entirely voluntary.

Alternatives to participation: If you choose not to participate, this will not affect your treatment in this hospital or receiving antenatal care in any way.

Withdrawal from the research: You may choose to withdraw from the research at any time without having to explain yourself.

Consequence of Withdrawal: There will be no consequence, loss of benefit or care to you if you choose to withdraw from the study.

Costs/Compensation: For your time/inconvenience/transport to the hospital, we will compensate you to show our appreciation for your participation

Contacts: If you have any question concerning this study, please do not hesitate to contact Dr. Christopher Larbie on 0243445961 or Miss. Deborah SakuaSackey on 0201517318/0549134113.

Further, if you have any concern about the conduct of this study, your welfare or your rights as a research participant, you may contact:

The Office of the Chairman
Committee on Human Research and Publication Ethics
Kumasi
Tel: 03220 63248 or 020 5453785

CONSENT FORM

Statement of person obtaining informed consent:

I have fully explained this research to _____ and have given sufficient information about the study, including that on procedures, risks and benefits, to enable the prospective participant make an informed decision to or not to participate.

DATE: _____ NAME: _____

Statement of person giving consent:

I have read the information on this study/research or have had it translated into a language I understand. I have also talked it over with the interviewer to my satisfaction.

I understand that my participation is voluntary (not compulsory).

I know enough about the purpose, methods, risks and benefits of the research study to decide that I want to take part in it.

I understand that I may freely stop being part of this study at any time without having to explain myself.

I have received a copy of this information leaflet and consent form to keep for myself.

NAME: _____

DATE: _____ SIGNATURE/THUMB PRINT: _____

Statement of person witnessing consent (Process for Non-Literate Participants):

I _____ (Name of Witness) certify that information given to

_____ (Name of Participant), in the local language, is a true reflection of what l have read from the study Participant Information Leaflet, attached.

WITNESS' SIGNATURE (maintain if participant is non-literate): _____

MOTHER'S SIGNATURE (maintain if participant is under 18 years): _____

MOTHER'S NAME: _____

FATHER'S SIGNATURE (maintain if participant is under 18 years): _____

FATHER'S NAME: _____