# ANTHROPOMETRY, BLOOD PRESSURE, FASTING BLOOD GLUCOSE, TOTAL CHOLESTEROL AND ELEMENTAL PROFILE OF PREGNANT WOMEN ATTENDING KIRU COMPREHENSIVE HEALTH CENTER KANO STATE

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## DECLARATION

I hereby declare that this work is the product of my own research efforts; undertaken under the supervision of (Prof. Hafiz Abubakar) and has not been presented and will not be presented elsewhere for the award of a degree or certificate. All sources of references have been duly acknowledged.

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## CERTIFICATION

This is to certify that the research work for this thesis and the subsequent preparation of this thesis by (Kabiru Tukur, SPS/08/SCI/00848) were carried out under my supervision.

_____ _____

Prof. Hafiz Abubakar Date
(Supervisor)

# APPROVAL PAGE

## DEDICATION

This work is dedicated to my loving and caring parents, may Jannatul Firdausi be our final abode.

## ACKNOWLEDGEMENT

All praise is to Allah the Almighty and the Lord of the Worlds, for His guidance throughout the period of my studies. May His bountiful blessing be upon his noble prophet Muhammad (S.A.W), his companions and all the members of his family.

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## ABSTRACT

The present study determined body weight and height (Body Mass Index-BMI-measurements were calculated according to the formula weight (kg)/height$^2$ (m), serum levels of Haemoglobin, Fasting Blood Glucose, Total Cholesterol and whole Blood levels of eight elements (calcium, cobalt, chromium, copper, iron, magnesium, lead and zinc in pregnant women (in the three trimesters) and in non-pregnant women. Sixty apparently healthy pregnant women and twenty non pregnant non lactating women (age range 17-34 years) participated in the study. The results showed significant difference in BMI of the pregnant women (1$^{st}$, 2$^{nd}$, and 3$^{rd}$ trimesters) and the control group ($p>0.05$). On the same being, the results indicated that there is a significant increase ($p<0.05$) in the Glucose and Total Cholesterol levels between the non pregnant women and pregnant women through the trimesters. Similarly, the Blood Pressure increases significantly as the pregnancy progresses. The results also showed that the levels of haemoglobin and that of the mineral elements assayed decreased significantly ($p<0.05$) as the pregnancy progressed through the three trimesters.

# CHAPTER ONE

# INTRODUCTION

## 1.10 BACKGROUND TO THE STUDY

Women are often more vulnerable to malnutrition than their male counterparts. This is particularly so in the developing countries where the larger population of the unemployed are women (Jacobs, 2000; Onimawo, 2001). Few of them who are employed have low per capita income. The vulnerability of women to malnutrition is heightened during conditions of pregnancy and lactation, periods which are characterized by increased nutritional needs. These nutritional needs include, a part from energy and protein, the requirement for micronutrients (minerals and vitamins), without which important metabolic pathways will be undermined. Among the essential mineral elements often required are iron (Fe) and calcium (Ca). Others are zinc (Zn), copper (Cu), magnesium (Mg), manganese (Mn), phosphorus (P), potassium (K), chlorine (Cl) and sodium (Na) (Onimawo, 2001).

Pregnant women together with young children and lactating women represent the group most vulnerable to nutritional deprivation. Most vulnerable because, their nutritional requirements are proportionally higher and the effects of malnutrition are severe and long lasting (Osofsky, 2005).

The end result of pregnancy, the new born, depends on its growth in uterus on the nutrients transferred from the mother, and its birth weight is dependent to a large extent to her nutritional status, not only during pregnancy but before it (Frisancho *et al.,* 1977).

Pregnancy and breastfeeding are the most nutritionally demanding times of a woman's life. The body needs enough nutrients every day to support the growth of the baby and the maintenance of the mother's body. All the nourishment which the developing baby needs comes from the mother, either through the foods she eats or the supplements she takes. Pregnant and lactating women need more essential nutrients than other women. The body needs an additional 300 calories each day to support the growth of the baby. It is important to eat the right foods every day since tissues and organs develop during certain weeks of pregnancy and the baby is always growing. Mother's health depends on diet, too. While the mother' body is supplying the nutrients the baby needs, her body still needs the same nutrients as before she was pregnant (Family and Consumer Sciences, 2009).

Women's nutrient needs increase during pregnancy and lactation. Some of the increased nutrient requirements protect maternal health while others affect birth outcome and infant health. If the requirements are not met, the consequences can be serious for women and their infants (Freedom from Hunger, 2003).

Adequate nutrition during pregnancy is extremely important to both maternal and fetal health. Dietary intake and gradual continuous weight gain in pregnancy are two of the most critical components of fetal growth and development (Wu *et al*., 2004). Nutritional requirements increase during pregnancy to support fetal growth and development as well as the increase in maternal metabolism and tissue development specific to reproduction (Picciano, 2003). Nutrients supplied to the fetus come from three sources—directly from

the mother's diet, from her nutrient stores, and from nutrient synthesis within the placenta (Guthrie and Picciano, 1995).

Adequate energy intake and a diversified diet that includes fruits, vegetables, and animal products throughout the life cycle help ensure that women enter pregnancy and lactation without deficiencies and obtain adequate nutrients during periods of heightened demand. Some nutrient requirements, particularly iron, folic acid, and vitamin A, are more difficult to achieve than others through food sources. For this reason, supplements with these nutrients are recommended in addition to improved diets. Fortified foods should be promoted through counseling and social marketing in countries where foods fortified with iron, iodine, folic acid, or vitamin A are available and affordable (Freedom from Hunger, 2003).

Intrauterine growth retardation (IUGR) of nutritional etiology can be a consequence of both low availability of nutrients from a malnourished mother and poor placental transfer of nutrients from a relatively nourished mother (Kramer, 1992). The environmental conditions that lead to poor maternal linear growth may also result in poor growth and suboptimal development of the anatomical and physiological system that sustain optimal fetal growth or maximize maternal health.

Improving adolescent's nutritional behaviors is an investment in adult health. Nutrition is only one aspect of health behaviors and the development of this in relation with chronic diseases is better conceptualized in a "chain of risk" frame work (Kuh *et al*., 1997). Over the life course there may be an accumulation of biological and social risk. For instance, poor fetal nutrition (Baker, 1996), in combination of inadequate or adverse influence of

parent, peers and educational experience in childhood and adolescence, set a stage for a chronic diseases in adult life.

It is suggested that the mechanism by which under nutrition delay or suppress activities of reproductive axis in adolescents or adult women, is through decrease available energy, rather than body composition changes (Cameroun, 1996 ) as suggested by reversal of exercise induced reproductive dysfunction by increasing food intake, without long-term negative effects on reproductive capacity.

Short stature is often time associated with small pelvises in women, and this is an important risk factor for obstructed labor. The risk rises sharply when the stature is below 1.45 cm, which is the case of 16-18 % of women in Asia, 11-15 % of women in Latin America and 3 % in Africa (ACC/SCN, 1992). So maternal stunting is a factor of increased obstetric risk and it could be attributed to chronic malnutrition, at least in part. In addition delayed growth and maturation in girls as a result of malnutrition further increases the risks associated with adolescent's pregnancy, as biological age lags behind chronological age (ACC/SCN, 1992).

### 1.11 Improving Nutrition throughout the Life Cycle

Pregnancy and lactation are times of heightened nutritional vulnerability. However, the threat of malnutrition begins in the womb and continues throughout the life cycle. A mother who was malnourished as a fetus, young child, or adolescent is more likely to enter pregnancy stunted and malnourished. Her compromised nutritional status affects the health and nutrition of her own children (WHO, 1998).

Growth faltering earlier in life leaves women permanently at risk of obstetric complications and delivering low birth weight babies. Deficiencies of some micronutrients, such as folic acid and iodine, affect the fetus shortly after conception .By the time the pregnancy is detected, permanent damage is done. For these reasons, maternal malnutrition cannot be addressed during pregnancy alone. The periods before and between pregnancies provide an opportunity for women of reproductive age to prepare for pregnancy by consuming an adequate balanced diet, including supplements and fortified foods where available, and by achieving a desirable weight (WHO, 1998).

Overweight and obesity at all ages, even in poor communities, present a difficult challenge for maternal and child health programs. Under weight and overweight often occur in the same communities and even the same households. Maternal overweight and obesity increase the risk of perinatal mortality, premature delivery, major birth defects, and maternal obstetric complications, including hypertension and gestational diabetes. Maternal and child health programs should alert women at all stages of the life cycle to the need to adjust diet and physical activity levels to achieve and maintain a desirable weight for their own health as well as for better birth outcomes ((Freedom from Hunger, 2003).

### 1.12 Anthropometry

Anthropometry deals with measurements of body sizes and proportions. Such measurements when put against age (e, g weight for age and height for age) or against each other (e. g weight for height) can yield good indicators of nutritional status (IITA, 2001). Anthropometric evaluation of nutritional status during the reproductive cycle,

particularly during pregnancy is widely use, low-technology procedure that may be expected to generate much valuable information (Wilcox and Horney, 1984). Unlike nutritional evaluation during other period of life which is concerned only with individual(s) in whom the measurements are made, measurements during pregnancy and lactation are expected to reflect both the nutritional status of the women and indirectly growth of fetus and later the quality and quantity of the breast milk (ACC/SCN, 1992).

Measurements taken early in pregnancy should be use to evaluate the nutritional status of the women and to predict how well she can cope with physiological demand of pregnancy. Unfortunately this objective is usually neglected, despite clear evidence that, in developing countries, pregnancy and lactation represent a major nutritional drain on the mother (Merchant and Mortorell, 1988). Measuring a women's height provides a proxy indicator of childhood growth and pelvic structure, and a good predictor of the risk of cephalopelvic disproportion and obstructed labor, which is a major cause of maternal death in developing countries (Krasovec and Anderson, 1991).

Assessment of maternal status during pregnancy is commonly based on height, weight, mid upper arm circumference and various measures of skin fold thickness. In addition maternal weight gain and fundal height may reflect fetal growth status. Height in adult is a reflection of the interaction of genetic potential for growth and environmental factors that influence the realization of such potential. Use of maternal height as an indicator of health and nutritional status must therefore take account of the environmental factors in which growth occurs (Krasovec and Anderson, 1991).

Use of anthropometry requires two essential items: an indicator and a cut-off point. The indicator often called anthropometric index, is measurement or a combination of measurements made in the field, such as weight and height, or the combination of measurement with additional data, such as age. Different indices reflect different component of nutritional status. The index weight for height indicates thinness, and because acutely under nourished person generally lose weight but not height, weight for height decreases with acute under nutrition (Bradley and Arabella, 2000).

Anthropometry can be used to evaluate either individual or population. To identify those in need of nutritional rehabilitation, a cut-off point is established below which persons are offered nutritional therapy. Anthropometry cannot provide a picture of the nutrition and food situation needed for problem solving and program planning. Anthropometry can provide an estimate of prevalence of under nutrition, but evaluation of food security, food distribution, nutrients content, morbidity and mortality and other elements are needed to understand the causal factors resulting in under nutrition (Young and Jaspars, 1995 ). For instance, a short stature or low pregnancy weights are additional risk factor. Height ranging from 140-150cm has been proposed as cut-off point for screening pregnant women with increase risk of intrauterine growth retardation and eventual complication at delivery (WHO, 1995).

Anthropometric indices (height and weight in case of children and body mass index (BMI) in case of adults) are widely used for assessment of adequacy of energy consumption. BMI, however, does not clearly bring out the entire extent of chronic under- nutrition. It has also been reported that body fat content for a given BMI is

different not only between men and women but also among different countries. BMI has been used to assess energy deficiency as well as excess. The currently used norms (<18.5 – under nutrition and >30 - obesity) were evolved on the basis of data from the developed countries where adverse health consequences of under nutrition have been shown to be associated with BMI values below 18.5 and the health hazards of obesity have been reported with BMI of over 30.

There are wide variations in height, weight, body composition and BMI right from birth through childhood and adolescence between countries and different income groups in the same country.

At the onset and any time during pregnancy, weight or BMI below the 25 % percentile of the reference value based on the result of WHO collective study has been suggested for screening pregnant women with increased risk of unfavorable pregnancy outcome. Routine measurement of weight is also recommended during pregnancy when resources permit in order to identify excesses or inadequate weight gain. As shown in the collaborative study (WHO, 1995), inadequate pre-pregnancy weight and inadequate weight gain have cumulative effects on risk of intrauterine growth retardation.

### 1.13 Multivitamin Use in Women

Adequate vitamin and mineral intake is important for the health of women of childbearing age, both for themselves and their infants. Deficiencies in some vitamins and minerals early in pregnancy can result in permanent damage to the fetus (Picciano,

2003). For example; supplements that contain folic acid decrease the risk of some birth defects (Correa *et al*., 2003).

Pregnancy increases the nutritional requirements for some vitamins and minerals, particularly iron. During pregnancy, many women do not get the daily recommended intake of micronutrients through dietary means (Pick *et al*., 2005). Several health organizations recommend iron supplementation during pregnancy to help pregnant women meet their iron requirements.

The Institute of Medicine recommends multivitamins that contain iron, zinc, copper, calcium, folic acid, and vitamins D, C, B6, and B12 for some groups of pregnant women, such as those with iron-deficiency anemia or poor-quality diets, vegetarians, cigarette smokers, and those who consume alcohol (Institute of Medicine, 1992).

## 1.20 NUTRITIONALLY ESSENTIAL ELEMENTS

Some metals are essential and their deficiency results in the impairment of the biological functions. There are three criteria for determining whether or not an element is essential;

- It must have direct influence on the organism and is involved in its metabolism
- The organism can never grow complete in its life cycle without adequate supply of the element and
- The element cannot wholly be replaced by any other (Alloway, 1990).

### Major Elements

Oxygen, hydrogen, carbon and nitrogen makes up just over 96 % of the human body mass that is the reason why they are called major elements. sodium, potassium, calcium, magnesium, phosphorus, sulphur and chlorine makes up 3.78 % of the body mass and the remaining 0.22 % of the body mass (about 70 elements) are called trace minerals. The terms minerals salts and macronutrients are used when the chemical occurs in a relatively higher or major quantities (express in milligrams/g) (Gomes and Silva, 2007).

### Trace Elements

The terms trace elements, oligoelements and micronutrients are used when chemical occurs in a minor or trace quantity (expressed in micrograms/g and comprises about 0.22% of the body mass (Gomes and Silva, 2007). Trace elements; zinc, manganese, cupper, iron, chromium and many others are necessary during pregnancy and these elements should be supplemented as a daily requirement in pregnant women (AMA, 1976).

## 1.3 SIGNIFICANCE OF SOME ELEMENTS

Metals such as sodium, potassium, calcium and magnesium are required by the body for its normal biological and physiological functions. Heavy metals in contrast are grouped into essential and non essential (Talwar *et al*., 1989). According to Amdur *et al.* (1991), chromium, cobalt, copper, magnesium and zinc are essential, but have potential for toxicity at higher concentration, while lead is non essential.

## 1.4 ROLES OF ELEMENTS IN DEFENCE AGAINST FREE RADICALS

The reactive oxygen produced during some biological processes in the living tissues can be damaging to those tissues and thus antioxidant mechanisms are necessary. Among these are the classical antioxidant vitamins, α-tocopherol (vitamin E) and ascorbic acid (vitamin C), glutathione a tripeptide composed of glutamine, cystine and glycine, antioxidant enzymes, superoxide dismutase and catalase, and the glutathione recycling enzymes, glutathione peroxidase. Superoxide dismutase has two forms; a mitochondrial form and a cytosolic form, the mitochondria form includes manganese at its active site, while the cytosolic form includes copper and zinc. Catalase contains iron at its active site, while glutathione peroxidase contains selenium (Hafiz, 2008).

## 1.5 DIABETES MELLITUS

Diabetes mellitus is a diagnostic term applied to a constellation of anatomic and biochemical abnormalities, which share a common, as part of a syndrome, a disturbance in glucose homeostasis, which is secondary to deficiency in the beta cells of the endocrine pancreas and characterized by the disorders of all kinds of metabolisms, especially carbohydrate metabolism (Patemkin, 1989). Diabetes mellitus occurs in 1-2 % of all pregnancies (Makowski, 1978).

The World Health Organization (WHO) expert committee (1995) on Diabetes Mellitus, the British Diabetic Association and the Working Party on Diabetes in Australia have agreed on criteria that the random venous plasma values of 11 mmol/l (198mg/dl) or more or fasting value of 8mmol/l (144mg/dl) or more are diagnostic of diabetes.

Akinyanju (1993) reported that in Nigeria, normal fasting blood sugar level lies between 45 mg and 100 mg (2.5 mmol-5.6 mmol) in 100 ml of blood.

## 1.6 GESTATIONAL DIABETES

The patient has no diabetes symptoms and had normal FBS but the 2-hour glucose tolerance test (GTT) reading is between 8 and 11 mmol/l. If this is found in pregnancy, it is referred to as gestational diabetes. Diabetes can develop for the first time when a woman becomes pregnant (Margaret, 1985). Usually the diabetes disappears after delivery but may reappear during the next pregnancy and in some the diabetes remains or continues even without pregnancy (Akinyanju, 1993). It occurs in about 3 % of pregnancies in developing countries (Darek, 1982).

## 1.7 STATEMENT OF THE PROBLEM

According to Paracelsus (1493 – 1541) "All substances are poison, there is none which is not a poison, the right dose differentiates a poison from a remedy" (Amdur *et al*., 1991). Although trace elements are of nutritional importance during pregnancy, the right dose may differ from organism to organism, but the sequences; deficiency, ideal concentration and toxicity are always the underlying principles (Abrahams *et al*., 2006).

Preeclampsia, a pregnancy specific disorder and a leading cause of maternal and prenatal mortality is associated with magnesium deficiency. Poor zinc status and changes in zinc metabolism during human pregnancy appear important since malformation of the offspring of zinc deficient animals have been reported (Warkany and Petering, 1972).

Copper and zinc are essential elements whose lacks give rise to deficiency diseases in plants and animals (Alloway, 1990).

It has been established that pregnant women with low calcium levels have a higher level of lead in their blood stream than those pregnant women with normal calcium levels and that lead is more likely to be released into the blood during the second half of pregnancy (Raviraja et al., 2008).

Iron is important in the diet of both pregnant women and nursing mothers, as well as the convalescents and the elderly to reduced cases of diseases associated with deficiency of iron such as anemia (Mohamed, 2000).

The number of pregnant women who are overweight or obese during pregnancy have also increased, more so obesity contributes to social, psychological and economical problems throughout a woman life time, which at times leads to cardiovascular diseases that affect both mother and fetus (Nyman, 2010).

## 1.8 JUSTIFICATION OF THE RESEARCH

Even though excess weight and obesity have gained attention as a serious threat to the global health, the assessment of nutritional status of pregnant women is not easy (Miguel, 1992).

During pregnancy excess weight increases both obstetric and neonatal risks, including gestational diabetes, high blood pressure, thrombosis, still birth and caesarean delivery. It is also more difficult to assess fetal development and evaluating fetal growth and position by external palpation (Nyman, 2010).

Man is composed of the major chemicals carbon, hydrogen, oxygen and nitrogen, the minerals salts and the trace minerals. Hence considering their physical and chemical properties, minerals can be essential to keep the human body in good shape, but in certain circumstances, deficiency or excess of mineral can be a factor of human disease generation (Gomes and Silva, 2007).

There are reports that preeclampsia and eclampsia are treated with magnesium in the hospital, and that calcium nutritive values in developing countries and in population other than Caucasian are poorly understood (WHO, 2005). Also reports on serum calcium levels are unclear, due to inadequate information on the effect of pregnancy on Black – Nigerian women (Idogun et al., 2007). Hence the need to assess the nutritional status of pregnant women cannot be over emphasised.

## 1.9 AIM AND OBJECTIVES

This research work is aimed at determining anthropometry, blood pressure, haemoglobin, fasting blood glucose, total cholesterol and some elemental profile as indices of nutritional status and their association with socioeconomic status among pregnant women attending Kiru Comprehensive Health Centre in Kano State.

The specific objectives of the study are:

a. Assessing the nutritional status of pregnant women using anthropometry and 24 hour dietary recall interview (using semi-structured interview scheduled).

b. To identify the possible statistical relationship between anthropometric and socioeconomic data as nutritional indicators.

c. To determine the levels of fasting blood glucose, total cholesterol, calcium, chromium, cobalt, copper, iron, magnesium and zinc of both pregnant and non pregnant women.

d. To determine the correlation of the different variables measured between the test (pregnant women) and the control group (non-pregnant women).

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1 MICRONUTRIENTS AND PREGNANCY OUTCOME

Women's nutrient needs increase during pregnancy and lactation. Some of the increased nutrient requirements protect maternal health while others affect birth outcome and infant health. If the requirements are not met, the consequences can be serious for women and their infants (WHO, 1998). The largest possible effects are noted on neonatal outcome in terms of low birth weight, preterm delivery, miscarriages and higher risks of morbidity in the first year of life. Evidence is accumulating that a deficiency in carotene, magnesium, zinc, vitamin C and possibly the B vitamins increase the risk of pre-eclampsia (Food and Nutrition Board, IOM, 2002).

Whether supplementation on a population based scale will yield improvements in pre-eclampsia incidence remains to be seen. Iron deficiency negatively affects birth weight, increases the chances of prematurity and it is highly likely that there are more complications during delivery for mother and child. Whether there is a direct effect on maternal mortality remains unclear. It is clear that supplements are needed in most regions where the prevalence of iron deficiency is high but it is not so clear whether supplementation is needed in situations where the prevalence is low (Stoltzfus and Dreyfuss, 1998).

During pregnancy the foetus is entirely dependent on the mother for growth and development. General physical status can therefore profoundly affect the health status

of the neonate at birth and survival chances. It is long known that a low energy intake during pregnancy affects birth weight. During the winter hunger in Holland (Lumey, 1988; 1992) and the siege of Leningrad (Antonov, 1947), at the end of the Second World War, food rations decreased considerably. Babies born in that period had a birth weight 338 gram lower than before the famine. These findings led to enthusiastic implementation of supplementary food programs during pregnancy in a wide variety of settings.

### 2.1.1 Pregnant women need more food, a varied diet, and micronutrient supplements.

When energy and other nutrient intake do not increase, the body's own reserves are used, leaving a pregnant woman weakened. Energy needs increase in the second and particularly the third trimester of pregnancy. Inadequate weight gain during pregnancy often results in low birth weight, which increases an infant's risk of dying. Pregnant women also require more protein, iron, iodine, vitamin A, folate, and other nutrients. Deficiencies of certain nutrients are associated with maternal complications and death, fetal and newborn death, birth defects, and decreased physical and mental potential of the child (Freedom from Hunger, 2003).

### 2.1.2 Adolescent's Nutritional Risk, Implications for Pregnancy

The greater the amount of uncompleted growth at conception, the greater the energy and nutrient needs above those normally required during pregnancy. Gynecologic Age (GA), the difference between chronological age and the age at menarche, can be used

as an indirect measure of physiologic maturity and growth potential. A pregnant adolescent with a GA of two years or less may still be in a period of growth and will have increased nutrient requirements compared to an adolescent who has finished her growth. Growth is generally completed four years after menarche (Story, 1992).

Research indicates that among young, growing pregnant adolescents, there may be competition for nutrients between the mother and fetus (Frisancho *et al*., 1984). This hypothesis was first put forth in 1981, by Naeye, who reported that fetuses grow more slowly in 10- to 16-year olds than in older women and speculated that there may be maternal-fetal competition for a limited supply of nutrients due to diminished placental blood flow(Naeye, 1981).

### 2.1.3 Preconception Nutritional Status

Attention has been placed on the importance of nutrition during preconception and the early months of pregnancy for healthy infant outcomes. For example, approximately 50% of cases of neural tube defects may be prevented with adequate intakes of folic acid from the time of conception and throughout the early months of pregnancy (Rayburn *et al*., 1996). A review of published studies of pregnant adolescents found that the nutrients consumed consistently in amounts substantially less than the RDA were: vitamins B6, D, E, and folate and the minerals iron, zinc, and magnesium. These same nutrients were also found to be low among more mature pregnant women (Institute of Medicine, 1990).

## 2.2 PREGNANT WOMEN'S IRON DIETARY REQUIREMENTS

Iron is important in diets for both pregnant and nursing mothers as well as the infants, the convalescents and the elderly to reduce cases of diseases associated with iron such as anemia (Mohamed, 2000). The consequences of anemia in term of poorer pregnancy outcomes are well known, in addition to a higher risk of low birth weight, prematurity, still birth, neonatal infection and maternal mortality. Anemia is the lack of adequate blood cell size or hemoglobin, causing inadequate transport of oxygen to all the cells in the body. Anemia in pregnancy may be associated with higher risk of hypertension and heart disease in the offspring of anemic mothers (Baker *et al*., 1990).

Iron deficiency anemia during the first two trimesters is associated with a twofold increased risk for preterm delivery and a threefold increased risk for delivering a low birth weight baby (Scholl *et al*., 1992). Iron requirements are further increased during pregnancy due to expansion of maternal plasma volume and growth of the fetus and placenta. The need for iron increases as pregnancy progresses. Iron needs increase throughout the second trimester reaching a peak requirement in the third trimester when fetal demands are greatest. The pregnancy Recommended Dietary Allowance for iron is 30 mg per day, a level twice that for non pregnant adolescents (Beard, 1994).

The provision of iron supplements in pregnancy is one of the most widely practiced public health measures, a high proportion of women in both industrialized and developing countries become anemic during pregnancy. Estimates are that 35% to 75% of pregnant women in developing countries and 18% of women in industrialized countries are anemic (WHO/FAO 1992). The prevalence of anemia in women increases

usually with 15 to 20% during pregnancy. A normal pregnancy needs an investment of 840 mg of iron with the highest needs in the second half. Iron is needed for the placenta, the increase in uterine size, expansion of the red blood cell mass and the fetus. The daily iron needs in the second half of pregnancy are estimated to be 6.7 mg per day even increasing up to 10-12 mg in the last month of pregnancy (Beard, 2000). With a normal average diet the absorption of non-haem iron needs to increase to 50% in order to cover the increased needs (Allen, 2000a). Absorption efficiency seems to increase during pregnancy but the studies vary considerably in estimates from 14.3% to 66% at 35 weeks gestation (Svanberg *et al*., 1976).

Consuming foods high in vitamin C along with plant (non-heme) sources of dietary iron enhances the body's ability to absorb iron. Iron deficiency anemia in pregnancy increases the risk of preterm birth and low-birth-weight and is also related to lower scores on intelligence, language, gross motor control, and attention tests in children at the age of five years (Tamura *et al*., 2002).

**Maternal Mortality and Anemia**

Allen (2000a) looked at the evidence for a relationship between maternal mortality and anemia. Most of the papers describing the relationship do so based on retrospective studies or on associations between hemoglobin levels and outcomes. In most of the studies the relationship was not corrected for health status, environment, nutritional status and health care provision. Many of the authors also believe that the relation of maternal mortality with anemia reflects the underlying pathology, which also causes anemia. This causality is more complex than iron deficiency alone and high prevalence

of an associated hookworm infection or megaloblastic anemia due to folic acid deficiency is usually diagnosed. Many of the authors concluded that mortality was not caused solely by anemia but that is was a contributing factor (Chi *et al*., 1981).

**Maternal Hemoglobin Concentrations and Birth Weight**

Changes in haemoglobin values during pregnancy make it difficult to determine anemia. The plasma volume changes during pregnancy and there is a drop in osmolarity, which reduces blood viscosity and enhances the blood flow in the intervillous space of the placenta. Enhanced blood flow improves foetal growth. Failure to reduce adequately blood viscosity with resulting high haematocrit values impairs foetal development (Story, 1992).

The relation between maternal anaemia and birth weight was reviewed extensively (Scholl and Hediger 1995; and Ramakrisham *et al*., 1999) and shows a U shaped relation with risk of low birth weight. Low and high haemoglobin values are associated with an increased risk of low birth weight, with an optimum range in between. Ramakrisham *et al*., (1999) analysed the haemoglobin values of 153,602 pregnancies collected in the Northwest Thames region (UK) between 1988 and 1991. The highest mean birth weight was found in association with a haemoglobin concentration of 85-95 g/L. The minimum incidence of low birth weight (<2.5 kg) and of preterm labour (< 37 weeks gestation) occurred in association with a haemoglobin concentration of 95-105 g/L. These values are below the WHO proposed cut-off values of 110 g/L, to define anaemia in pregnant women (Hallberg, 1988). Lower and higher haemoglobin values increase the odds for low birth weight in a dose related fashion (Dreyfuss, 1998)

### Maternal Iron Deficiency Anaemia and Duration of Gestation

This issue of preterm delivery was extensively reviewed in a number of reviews (Scholl and Hediger, 1995; Ramakrisham *et al*., 1999; Allen 2000a; 2000b). There seems a consistency in the findings that iron deficiency anemia, but not other forms of anemia, increase the risk of preterm deliveries and associated low birth weight. Allen (2000a) concludes: The results are consistent with an association between maternal iron deficiency anemia in early pregnancy and a greater risk of preterm delivery. The association disappears in the third trimester of pregnancy.

## 2.3 PREGNANT WOMEN'S ZINC DIETARY REQUIREMENTS

Zinc is essential for the growth and development of human life and has an active role in the body function. During pregnancy zinc is also used to assist the fetus to develop the brain and also to be an aid to the mother in the first and second stages of labour (Nyman *et al*., 2010). It is also an essential component of more than two hundred enzymes involved in digestion, metabolism, reproduction, and wound healing (Gomes and Silva, 2007).

Furthermore, Caulfield *et al*., (1998) reported that strong association has been observed between poor maternal zinc status and various indicators of poor pregnancy outcomes, although supplementation trials results are still needed to define the public health importance of zinc deficiency worldwide. Zinc also plays a role in the biosynthesis of enzymes and proteins, bone development, wound healing and reproduction. Zinc

deficiency syndrome as demonstrated in man is characterized by growth retardation or failure, skin lesion and defects in spermatogenesis (Talwar *et al*., 1989).

Studies conducted by van Wouwe (1989), of experimental animals and in humans indicate that severe zinc deficiency can have profound effects on pregnancy outcome. Severe zinc deficiency causes prolonged labour, teratogenesis, and embryonic or foetal death. Acrodermatitis enteropathica is an autosomal genetic recessive defect in zinc metabolism and causes a marked inhibition of zinc absorption (Van Wouwe, 1989). The outcomes of pregnancies with acrodermatitis enteropatica ended in spontaneous abortion, anencephaly, achondroplastic dwarfism and low birth weight infants (Hambidge *et al*., 1975). When these patients were given high dosages of oral zinc to maintain normal plasma zinc concentrations throughout gestation, pregnancy outcomes were normal.

Zinc affects protein synthesis and is essential for growth. The recommended intake during pregnancy is 15 mg of zinc per day. Young pregnant teenagers who are still growing may have higher needs. In one study, low intakes of zinc were associated with an increased risk of low birth weight and preterm delivery in adolescents. Zinc requirements are highest in the third trimester when the fetus acquires two-thirds of its zinc stores (Scholl and Hediger, 1995).

Several studies (King, 2000, Caulfield *et al*., 1998 and Van Wouwe, 1989) have documented the relation between maternal zinc status and pregnancy outcome. The results are mixed and several adverse effects have been associated with low zinc status. These include congenital anomalies, reduced birth weight for gestational age and

preterm delivery. Maternal complications include pregnancy-induced hypertension, preeclampsia, intrapartum haemorrhage, infections, and prolonged labour.

A review (Tamura and Goldenberg, 1996) analysed 41 studies of maternal zinc status and birth weight published between 1977 and 1994. Seventeen of the 41 studies recorded a significant relation between indicator of maternal zinc status and birth weight.

## 2.4 PREGNANT WOMEN'S MAGNESIUM DIETARY REQUIREMENTS

The contribution of the mineral magnesium to overall health has been largely over looked until recent years. The latest research suggest that magnesium may play a vital role in facilitating healing and promoting health in the human body in conditions as diverse as fibromyalgia and diabetes. Studies conducted over the past ten years suggests that magnesium plays a role in preventing heart disease and cancer, relieves the symptoms of asthma and reduced emotional irritability in cases of chronic depression (Lucas *et al*., 1995). It also activates over hundred enzymes and helps in nerves and muscle function (Gomes and Silva, 2007). Magnesium supplementation studies shows a lower incidence of low birth weight due to intrauterine growth retardation, but some methodological weaknesses may hamper generalization of the findings (Gulmezoglu *et al*., 1997).

Magnesium is an essential mineral needed in relatively large amounts by humans. In a number of retrospective studies magnesium levels during pregnancy were found to be associated with the risk of seizures in pre-eclampsia, prematurity and low birth weight

(Ramakrisham *et al*., 1999; Makrides and Crowther, 2000). This promising association has triggered a number of controlled supplementation studies, which have been reviewed lately (Makrides and Crowther 2000).

## 2.5 PREGNANT WOMEN'S CALCIUM DIETARY REQUIREMENTS

Calcium is essential for the development and maintaining healthy bones and teeth, assist in blood clotting, muscle contraction and nerves transmission. Its supplementation during pregnancy was seen to reduce the risk of pre-term delivery (but not impaired fetal growth) and of preeclampsia and pregnancy induced hypertension, according to systematic reviews of controlled trials (Gulmezoglu *et al*., 1997).

Calcium is needed in infants for strong bones. If calcium is not supplied through the mother's diet, it is taken from the mother. Pregnant and breastfeeding women 19 years and older need around 1,000 milligrams per day while 1,300 milligrams per day are needed for women under 19 years of age. Calcium absorption in women increases two-fold during pregnancy (Guthrie and Picciano, 1995).Consequently; the calcium requirements of pregnant women are similar to those of non-pregnant women. In addition, women with pregnancy-induced hypertension may also benefit from higher calcium intakes (Katz, 2001).

During pregnancy, approximately 25-30 g of calcium is transferred to the fetus. Most of this is acquired during the third trimester, when calcium is deposited into the fetal skeleton at a rate of about 330 mg per day. In an adult woman, this represents about 2.5% of typical maternal bone calcium stores (Institute of Medicine, 1990). Few studies

indicate that pregnant adolescents may benefit from a high calcium intake (Institute of Medicine, 1997). A lower incidence of preterm delivery and low birth weight was observed in pregnant adolescents randomized to receive 2000 mg per day supplemental calcium (Villar and Repke, 1990).

High blood pressure with or without proteinuria is a major cause of maternal and perinatal morbidity and mortality worldwide. Preterm birth, a common association with hypertensive disorders, is the leading cause of early neonatal death and infant mortality, particularly in low income countries. A number of observation studies led to the hypothesis that an increase in calcium intake during pregnancy might reduce the incidence of high blood pressure and preeclampsia among women with low calcium intake (Atallah *et al*., 2000).

There was no evidence that supplements decreased preterm delivery, although there was a reduction in risk among women at risk of hypertension. There was no effect of calcium supplementation on stillbirth or death before discharge from the hospital, but there were fewer babies with a low birth weight (Herrera et al., 1998, Crowther *et al*., 1999).

## 2.6 PREGNANT WOMEN'S CHROMIUM DIETARY REQUIREMENTS

Chromium is widely distributed in low variable concentration. Unlike most trace elements, the tissue level of chromium declines with age. It was described to have growth promoting effect, thus recognized as an essential trace element for humans. Even though it has not been assigned a Recommended Dietary Allowance value by the

United State National Research Council, an estimate safe and adequate daily intake of 50-200μg for adults has been set (Jan Alexander, 1993). Chromium aids in glucose and lipid metabolism and regulation of blood sugar, but the mechanism of action and functional molecular role of chromium in intermediary metabolism in human are still unclear. Chromium is important for glucose tolerance, and chromium responsive insulin resistance has been reported (Jan Alexander, 1993).

## 2.7 PREGNANT WOMEN'S COBALT DIETARY REQUIREMENTS

Cobalt is a transition metal that exists in +2 or +3 oxidation state. The first indication that cobalt is an essential element came in 1935. It is an essential compound of vitamin B12 otherwise called antiperniciuos anemic factor of which 4 % is cobalt (Talwar *et al*., 1989). The significance role of cobalt in human nutrition is confined to the presence in vitamin B12. It also promotes the formation of red blood cells (Gomes and Silva, 2007).

## 2.8 PREGNANT WOMEN'S COPPER DIETARY REQUIREMENTS

Copper is widely distributed in the human body with content of about 60-100mg. it is required more in new born than in adults, with serum level being shortly higher in female than male (Talwar *et al*., 1989). It also promotes the formation of red blood cells and connective tissues, acts as a catalyst to store and release iron to help forms hemoglobin, contributes to central nervous system function and the synthesis of adrenal hormones (Gomes and Silva, 2007). It is required for the function of certain enzymes such as catalase, peroxidase and cytochrome oxidase (Amdur *et al*., 1991). Copper also

prevent anemia and interrelated with function of zinc and iron in the body (Mohamed and Hytten, 1982).

## 2.9 Some of the Factors Leading to Obesity

### Diet and Physical Inactivity

Poor diet and physical inactivity are the most influential factors contributing to the increase in overweight and obesity in the United States. Pregnant women and women of child bearing age are not immune to these factors (USDHHS and USDA, 2005). Diet counseling during pregnancy should ensure that energy and nutrient intake is neither excessive nor deficient (Katz, 2001).

### Eating food away-from-home

A small study of 150 women in Texas suggests that eating food away from home during pregnancy contributes to a higher intake of energy, total fat, and saturated fat. (George *et al*, 2005).

### Food Security

Access to enough food at all times defines food security while food insecurity refers to a household with limited or uncertain availability of food. Achieving adequate nutrition is dependent on a number of socioeconomic factors including, age, family income, social status, ethnicity, education, employment, marital status, and availability of healthcare and support systems (Mitchell, 2003). In a recent study among pregnant women, income level was the characteristic most predictive of food security. The study

also found that life stress and coping behaviors may be as important as income in determining an individual's risk for food insecurity (Laraia *et al*., 2006).

**Media**

Conflicting messages from the media, government, industry, health care professionals, family, and friends can adversely affects the nutritional status of a population. According to a 2003 Food Marketing Institute survey the top seven sources for consumers seeking information about health and nutrition include: healthcare professionals, books, magazines, family/friends, newspapers, television, and the Internet (FMI Shopping for Health*, 2003)*.

## 2.10 GESTATIONAL DIABETES

Pregnant women who have never had diabetes before but who have high blood glucose levels during pregnancy have gestational diabetes (GDM). GDM complicates approximately 7 percent of all U.S. pregnancies annually, resulting in about 200,000 cases per year. Of these women, 20 – 50 percent has a chance of developing type 2 diabetes in the five to ten years following their pregnancy. The most common prenatal risk of gestational diabetes is fetal macrosomia (ADA, 2004).

Gestational Diabetes is more common in women who; have a first degree relative with diabetes, are obese, are from one of the following ethnic groups; American Indian, African American, Hispanic, Asian/Pacific Islander, have had a previous baby weighing more than nine pounds, had a previous baby that died before birth (stillbirth),

have polycystic ovarian syndrome and have chronic use of medications that increase the risk of diabetes (e.g. steroids) (CDPPDC, 2006).

A report of Crowther *et al*. (1999) confirming the efficacy of screening for and treatment of mild-to moderate levels of glucose intolerance in mid-pregnancy in reducing both perinatal and maternal morbidity has set the stage for universal maternal screening and there by identifies a cohort of young women (as many as 200,000 annually in the U.S.) who may be at risk for subsequent hypertension and vascular disease.

## 2.11 GESTATIONAL HYPERTENSION (GH)

GH was defined as a systolic BP of at least 140 mm Hg and/or a diastolic BP of at least 90 mm Hg on at least two occasions at least 6 hours apart after the 20th week of gestation in women known to be normotensive before pregnancy and before 20 weeks' gestation. The BP recordings used to establish the diagnosis should be no more than 7 days apart (NHBPEP, 2000). Gestational hypertension is considered severe if there is a sustained elevation in systolic BP to at least 160 mm Hg and/or in diastolic BP to at least 110 mm Hg for at least 6 hours (AOOG Committee, 2001).

Gestational hypertension is the most frequent cause of hypertension during pregnancy. The rate ranges between 6% and 17% in healthy nulliparous women and between 2% and 4% in multiparous women (Hnat *et al*., 2002). The rate is further increased in women with previous preeclampsia and in women with multifetal gestation. Some women with gestational hypertension will subsequently progress to preeclampsia. The

rate of progression depends on gestational age at time of diagnosis; the rate reaches 50% when gestational hypertension develops before 30 weeks of gestation (Barton *et al*., 2001).

**Classification of Gestational Hypertension**

The classification of the hypertensive disorders of pregnancy is based on the two most common manifestations of preeclampsia: hypertension and proteinuria. Gestational hypertension and preeclampsia are common disorders during pregnancy, with the majority of cases developing at or near term. The development of mild hypertension or preeclampsia at or near term is associated with minimal maternal and neonatal morbidities. In contrast, the onset of severe gestational hypertension and/or severe preeclampsia before 35 weeks' gestation is associated with significant maternal and perinatal complications (Yates *et al*., 1998).

Approximately 70% of women diagnosed with hypertension during pregnancy will have gestational hypertension–preeclampsia. The term "gestational hypertension–preeclampsia" is used to describe a wide spectrum of patients who may have only mild elevation in blood pressure (BP) or severe hypertension with various organ dysfunctions including acute gestational hypertension; preeclampsia; eclampsia; and hemolysis, elevated liver enzymes, low platelets (HELLP) syndrome. The exact incidence of gestational hypertension–preeclampsia in the United States is unknown. Estimates range from 6% to 8% of all pregnancies (Yates *et al*., 1998).

**Pre-eclampsia.**

Pre-eclampsia, a multisystem disorder of pregnancy usually associated with raised blood pressure and proteinuria, complicates 2–8% of pregnancies (Hallberg, 1988). Preeclampsia is primarily defined as gestational hypertension plus proteinuria (300 mg or more per 24-hour period). If 24-hour urine collection is not available, then proteinuria is defined as a concentration of at least 30 mg/dL, in at least two random urine samples collected at least 6 hours apart (Yates *et al*., 1998). Severe proteinuria is defined as protein excretion of at least 5 g per 24-hour period. Although outcome is often good, pre-eclampsia is a major cause of morbidity and mortality for the woman and her child (NICE, 2001).

Eclampsia is defined as the occurrence of one or more convulsions superimposed on pre-eclampsia. In developed countries eclampsia is rare, affecting around one in 2000 deliveries (Douglas and Redman, 1994), while in developing countries estimates vary from one in 100 to one in 1700 (Crowther, 1985). Worldwide an estimated 600 000 women die each year of pregnancy-related causes (WHO and UNICEF, 1996), with 99% of these deaths occurring in developing countries. Pre-eclampsia and eclampsia probably account for more than 50000 maternal deaths a year (Duley, 1992). In places where maternal mortality is high, most of these deaths are associated with eclampsia. Where maternal mortality is lower, a higher proportion will be due to pre-eclampsia. For example, in the UK pre-eclampsia and eclampsia together account for 15% of direct maternal deaths, and two-thirds were related to pre-eclampsia (NICE, 2001).

Pre-eclampsia refers to a set of symptoms rather than any causative factor, and there are many different causes for the condition. It appears likely that there are substances from the placenta that can cause endothelial dysfunction in the maternal blood vessels of susceptible women (Drife, 2007).

Pre-eclampsia may develop from 20 weeks gestation (it is considered early onset before 32 weeks, which is associated with increased morbidity). Its progress differs among patients; most cases are diagnosed pre-term. Pre-eclampsia may also occur up to six weeks post-partum. Apart from Caesarean section or induction of labor (and therefore delivery of the placenta), there is no known cure. It is the most common of the dangerous pregnancy complications; it may affect both the mother and the unborn child (Drife, 2007).The hypertensive disorders of pregnancy are a leading cause of maternal and perinatal mortality and morbidity in Canada (Health Canada, 2004), and internationally (Roberts *et al*, 2003).

The rate of preeclampsia ranges between 2% and 7% in healthy nulliparous women (Knuist *et al*, 1998). The rate is substantially higher in women with twin gestation (14%) (Sibai *et al*, 2000) and those with previous preeclampsia (18%) (Hnat *et al* 2002).

The management of preeclampsia includes medications to reduce high blood pressure, strict bed rest, maintenance of normal salt intake, possibly hospitalization and induced delivery or caesarean section. Despite the improved health care worldwide, preeclampsia and eclampsia continue to take a great toll on life of pregnant women

especially in rural Nigerian communities where obstetric interventions may be lacking or inadequate (Idogun *et al*., 2007).

**Diagnosis of Preeclampsia**

Pre-eclampsia is diagnosed when a pregnant woman develops high blood pressure (two separate readings taken at least six hours apart of 140 or more in systolic blood pressure and/or 90 or more in diastolic blood pressure) and 300 mg of protein in a 24-hour urine sample (proteinuria). Swelling or edema (especially in the hands and face) was originally considered an important sign for a diagnosis of pre-eclampsia, but in current medical practice only hypertension and proteinuria are necessary for diagnosis. Pitting edema (unusual swelling, particularly of the hands, feet, or face, notable by leaving an indentation when pressed on) can be significant for diagnosis. Severe pre-eclampsia involves a BP over 160/110 (mmHg) and additional symptoms (Laura et al., 2008).

Pre-eclampsia may progress to eclampsia, characterized by the appearance of tonic-clonic seizures. This happens only very rarely with proper treatment. Although eclampsia is potentially fatal (20% of cases), pre-eclampsia is often asymptomatic, and so its detection depends on signs or investigations. In general, none of the signs of pre-eclampsia are specific, and even convulsions in pregnancy are more likely to have causes other than eclampsia in modern practice. Diagnosis, therefore, depends on finding a coincidence of several pre-eclamptic features, the final proof being their regression after delivery (Laraia *et al*., 2006).

## Epidemiology of Preeclampsia

Pre-eclampsia affects approximately 6-8% of all pregnancies worldwide (WHO, 2005), with onset of symptoms in the late second or third trimester, most commonly after the 32$^{nd}$ week. Some women will experience pre-eclampsia as early as 20 weeks, though this is rare. It is much more common in women who are pregnant for the first time (Robbins and Cotran, 2008), and its frequency drops significantly in second pregnancies. While change of paternity in a subsequent pregnancy is now thought to lower risk except in those with a family history of hypertensive pregnancy (Hjartardottir *et al*., 2004). Since increasing maternal age raises risk, (Zhang, 2007) it has been difficult to evaluate how significant paternity change actually is and studies are providing conflicting data on this point.

Pre-eclampsia may also occur in the immediate post-partum period. This is referred to as postpartum pre-eclampsia. The most dangerous time for the mother is the 24–48 hours postpartum and careful attention should be paid to pre-eclampsia signs and symptoms (Munjuluri *et al*., 2005).

## Causes of Preeclampsia

The pre-eclampsia syndrome is thought in many cases to be caused by a shallowly implanted placenta which becomes hypoxic, leading to an immune reaction characterized by secretion of up regulated inflammatory mediators from the placenta, and acting on the vascular endothelium. The shallow implantation is thought to stem from the maternal immune system's response to the placenta. This theory emphasizes

the role of the maternal immune system, and refers to evidence suggesting a lack of established immunological tolerance in pregnancy, resulting in an immune response against paternal antigens from the fetus and its placenta (Burne and Jerome, 2006).

In some cases of pre-eclampsia it is thought that the mother lacks the receptors for the proteins the placenta is using to down regulate the maternal immune system's response to it (Moffett and Hilby, 2007). This view is also consistent with evidence showing many miscarriages to be an immunological disorder where the mother's immune system unleashes a destructive attack on the tissues of the developing child (Immune system, 2000).

Many theories have attempted to explain why pre-eclampsia arises, and have linked the syndrome to the presence of the following; dietary factors, including vitamin deficiency and genetic factors (Courtney *et al*., 2006), air pollution and obesity (Jun *et al*., 2009). Thyroid dysfunction: Subclinical hypothyroidism in early pregnancy, compared with normal thyroid function, has been estimated to increase the risk of pre-eclampsia with an odds ratio of 1:7 (Boogaard *et al*., 2011).

**Pathogenesis of Preeclampsia**

Although much research into the etiology and mechanism of pre-eclampsia has taken place, its exact pathogenesis remains uncertain. Some studies support notions of inadequate blood supply to the placenta making it release particular hormones or chemical agents that, in mothers predisposed to the condition which leads to damage of

the endothelium (lining of blood vessels), alterations in metabolism, inflammation, and other possible reactions (Drife, 2007, Van *et al*., 2011).

Abnormalities in the maternal immune system and insufficiency of gestational immune tolerance seem to play major roles in pre-eclampsia. One of the main differences found in pre-eclampsia is a shift toward Th1 responses and the production of interferon (IFN-ã). The origin of IFN-ã is not clearly identified and could be the natural killer cells of the uterus, the placental dendritic cells modulating responses of T helper cells, alterations in synthesis of or response to regulatory molecules, or changes in the function of regulatory T cells in pregnancy (Laresgoiti-Servitge *et al*., 2010). Aberrant immune responses promoting pre-eclampsia may also be due to an altered fetal allorecognition or to inflammatory triggers (Laresgoiti et al., 2010). It has been documented that fetal cells such as fetal erythroblasts as well as cell-free fetal DNA are increased in the maternal circulation in women who develop pre-eclampsia. These findings have given rise to the hypothesis that pre-eclampsia is a disease process by which a placental lesion such as hypoxia allows increased fetal material into maternal circulation that leads to an immune response and endothelial damage ultimately resulting in pre-eclampsia and eclampsia (Jun *et al*., 2009).

**Complications of Preeclampsia**

Eclampsia can occur after the onset of pre-eclampsia. Eclampsia, which is a more serious condition, complicates 1 in 2000 maternities in the United Kingdom and carries a maternal mortality of 1.8 percent (Douglas and Redman, 1994). The HELLP syndrome is more common, probably about 1 in 500 maternities, but may be as

dangerous as eclampsia itself. These two major maternal crises can present unheralded by prodromal signs of pre-eclampsia. Cerebral hemorrhage is a lesion that can kill with pre-eclampsia or eclampsia. In that cerebral hemorrhage is a known complication of severe hypertension in other contexts, it must be assumed that this is a major predisposing factor in this situation, although this has not been proven (Burne and Jerome, 2006). Uric acid levels may help to predict maternal complications among patients with pre-eclampsia according to a systematic review and decision analysis (Koopmans *et al*., 2009).

## 2.12 MAGNESIUM IN PRE-ECLAMPSIA AND ECLAMPSIA

Eclampsia was noted to be associated with albuminuria in 1839 and with hypertension in 1897. These discoveries, coupled with the introduction of prenatal care in the first decade of the 20th century, led to earlier diagnosis of preeclampsia. Despite earlier diagnosis and safer cesarean operations, however, maternal mortality due to eclampsia in the United States remained at 25 to 30 percent through the first quarter of the 20th century. Success in treating tetanus with intraspinal magnesium sulfate in 1906 soon led to its intravenous use in women with eclampsia in Los Angeles. By 1929, the introduction of intramuscular magnesium sulfate treatment at the Chicago Lying-In Hospital was associated with a reduction in the maternal mortality rate from 36 percent to 7 percent. Subsequently, in large consecutive case series of patients with eclampsia in the United States from 1955 through the 1980s, maternal deaths were virtually eliminated among women treated empirically according to standardized protocols in which magnesium sulfate was the cornerstone of therapy (Michael and Greene, 2003).

The use of magnesium sulphate for pre-eclampsia is increasing (Gulmezoglu and Duley, 1998). In the USA, for example, magnesium sulphate is given to an estimated 5% of pregnant women before delivery (Lucas *et al*., 1995).

The consequences of low magnesium may lead to a reduction in cerebral blood flow, cerebral vasospasm and increase in neuronal burst. Macdonald *et al* (2004) have shown experimentally that magnesium has a vasoprotective effects. And this explains the use of magnesium sulphate as a neuroprotectant and antivasospastic agent. Magnesium ($Mg^{++}$) may increase cerebral blood flow and reduces the contraction of cerebral arteries caused by various stimuli (Macdonald *et al*., 2004). The systemic effects of magnesium include vasodilatation and an increase blood flow, these prevent eclampsia by selectively dilating the cerebral vasculature and relieving the cerebral vasospasm associated with preeclampsia. In the eclamptic patient, it will prevent a recurrent seizure which is beneficial in reducing the mortality and morbidity in both mother and fetus (Shahnaz *et al*., 2007).

The aetiology of pre-eclampsia and eclampsia remains elusive. Exactly how magnesium sulphate might control eclamptic convulsions is also unclear. Magnesium may have a localized cerebral effect. For example, it may cause vasodilatation with subsequent reduction of cerebral ischaemia (Belfort and Moise, 1992), and/or block some of the neuronal damage associated with ischaemia (Goldman and Finkbeiner, 1988). Alternatively, any effects of magnesium sulphate in control of eclamptic convulsions may be, wholly or part, through its role as a blocker of N-methyl-D-aspartate receptors in the brain (Sadeh, 1989). These receptors are activated in response

to asphyxia, leading to calcium influx into the neurons, which causes cell injury. It is suggested that magnesium may block these receptors, so reducing calcium influx and protecting the neurons from damage (Sadeh, 1989). Magnesium sulphate is remarkably effective at reducing the risk of eclampsia, whether this is the first seizure or recurrence of convulsions (The Eclampsia Trial Collaborative Group, 1995).

Belfort and Moise (1992) reported that magnesium sulfate was clearly superior to the selective cerebral vasodilator nimodipine in reducing the risk of seizures in women with preeclampsia. At the beginning of the 21st century, the mechanism of action of the simple magnesium ion in preeclampsia and eclampsia eludes precise definition, yet magnesium remains the standard of care (Michael and Greene, 2003).

## 2.13 CALCIUM IN PREECLAMPSIA AND ECLAMPSIA

Calcium ions have been implicated in seizure disorders, which often complicate preeclampsia (Idogun *et al*., 2007). Calcium and magnesium are two intracellular ions that are very important for cellular metabolism such as muscle contractility, secretions, neuronal activity as well as cellular death (Vinay *et al*., 1999). While the causes of preeclampsia remain elusive to scientific knowledge, magnesium and calcium deficiencies are thought to be implicated (David and Robert, 1999). Studies from the early 1950s first elucidated the nature of the effects of calcium and magnesium ions at the neuromuscular junctions. Magnesium competes for a prejunctional site with calcium ions. The ions competed with each other; high magnesium concentrations inhibit the release of acetylcholine (Ach) and high calcium concentration increases release of Ach from pre-synaptic nerve terminal. During cellular injury and cellular

death, there is influx of calcium ions into the cell leading to increased intracellular calcium ions and loss of calcium homeostasis (Fawett *et al*., 1999).

Calcium supplementation during pregnancy may prevent high blood pressure and preterm labor. Calcium supplementation appears to be beneficial for women at high risk of gestational hypertension and in communities with low dietary calcium intake. (Hofmeyr *et al*., 2004).

An inverse relationship between calcium intake and hypertensive disorders of pregnancy was first described in 1980 (Belizan and Villar, 1980). This was based on the observation that Mayan Indians in Guatemala, who traditionally soak their corn in lime before cooking, had a high calcium intake and a low incidence of preeclampsia and eclampsia. A very low prevalence of preeclampsia had been reported from Ethiopia where the diet, among other features, contained high levels of calcium (Hamlin, 1962). These observations were supported by other epidemiological and clinical studies (Hamlin, 1952, Belizan and Villar, 1980), and led to the hypothesis that an increase in calcium intake during pregnancy might reduce the incidence of high BP and pre-eclampsia among women with low calcium intake (Hofmeyr *et al*., 2004).

Low calcium intake may cause high BP by stimulating either parathyroid hormone or renin release, thereby increasing intracellular calcium in vascular smooth muscle (Belizan *et al*., 1988) leading to vasoconstriction. A possible mode of action for calcium supplementation is that it reduces parathyroid release and intracellular calcium, and so reduces vascular smooth-muscle contractility. By a similar mechanism, calcium supplementation could also reduce uterine smooth-muscle contractility and prevent

preterm labour and delivery (Villar and Repke, 1990). Calcium might also have an indirect effect on smooth-muscle function by increasing magnesium levels (Repke *et al*., 1989).

A systematic review by Hofmeyr *et al*. (2004) revealed that calcium supplementation was associated with: reduced hypertension, reduced pre-eclampsia, particularly for those at high risk and with a low baseline dietary calcium intake, reduced low birth weight (for women at high risk of hypertension), reduced preterm delivery, and reduced childhood hypertension. No side-effects of calcium supplementation have been recorded in the trials reviewed.

# CHAPTER THREE

# MATERIALS AND METHODS

## 3.1 STUDY AREA

Kano is one of the states that make up the region called Northern Nigeria. The global location of the state is between longitude $12^{o}$ $03^{1}$ east of the Greenwich and also between latitude $08^{o}$ $32^{1}$ north of equator (Japheth, 2002). The state occupies an area approximately 60,473.2 $km^2$ and has projected population of 9,401,288 (National Population Commission, 2006). Kiru Local Government is located within latitudes $11^{o}20^{1}$N to $11^{o}$ $45^{1}$N and longitude $7^{o}$ $58^{1}$ E to $8^{o}$ $16^{1}$ E. It shares political boundaries to the North with Kabo, North-East with Madobi, East with Bebeji, South-East with Tudun Wada, South – West with Kaduna State and North West with Karaye Local Government Area. Kiru Local Government covers an area of 1000 $km^2$ and is predominantly inhibited by Hausa Fulani. Going by the 2006 population census the area is inhibited by 264,781 peoples (Abdullahi, 2007).

## 3.2 DESIGN AND ADMINISTRATION OF THE QUESTIONNAIRE

Socio-demographic data of the participants were collected by means of a semi-structured interview schedule (Appendix I). Informants were participants under study.

## 3.3 DATA COLLECTION

Ethical approval was sought from the hospital, through Kiru Local Government Council. Pregnant and non pregnant women aged between 15-40 years attending Kiru Comprehensive Health Center were interviewed and blood samples were collected from those who gave their written informed consent (Appendix II).

## 3.4 PRELIMINARY INVESTIGATION

The selected Hospital was visited first, to discuss with officials on what the study was all about. The consent and cooperation of the officials and the host community were obtained.

## 3.5 SAMPLE COLLECTION

A total of 80 blood samples were collected 60 from pregnant women, 20 each from the 3 trimester respectively, while the remaining 20 from non pregnant/non lactating women attending Kiru Comprehensive Health Center. Fasting venous blood sample (5ml) was collected from each participant, using plastic disposable syringes. Each sample was divided into two, one portion was transferred into a sterilized lithium heparin container, carefully mixed and refrigerated in the laboratory, while serum was separated from the other portion and transferred into appropriately labeled sample bottles. Also for test involving serum, as much as possible, samples were analysed on the day of collection. The participants were also interviewed for anthropometrical variables (weight and height) that were measured according to standard techniques and calibrated instruments.

## 3.6 REAGENTS

**Table 1: Chemical and Reagents**

| Chemical | Formula | Grade | Manufacturer |
|---|---|---|---|
| Conc. HCl acid | HCl | Analytical | BDH – Analar |
| Conc. Sulphuric acid | $H_2SO_4$ | " | " |
| Conc. Nitric acid | $HNO_3$ | " | " |
| Calcium Carbonate | $CaCO_3$ | Laboratory Chemical | May & Baker |
| Potassium dichromate | $K_2Cr_2O_7$ | " | " |
| Cobalt chloride | $CoCl_2.6H_2O$ | " | " |
| Iron sulphate | $FeSO_4.7H_2O$ | " | " |
| Magnesium sulphate | $MgSO_4.7H_2O$ | " | " |
| Lead nitrate | $Pb(NO_3)_2$ | " | " |
| Zinc sulphate | $ZnSO_4.7H_2O$ | " | " |
| Sodium Chloride | NaCl | " | " |
| 4-aminophenazone | | Analytical | BDH-Analar |
| Sodium Acetate | $CH_3COO$-Na | Lab Chemical | May & Baker |
| Fercozyme | | Analytical | BDH-Analar |
| Glacial Acetic acid | $CH_3COOH$ | Lab Chemical | May & Baker |
| Glucose | | " | May & Baker |
| Ferric Chloride | $FeCl_3$ | " | May &Baker |
| Cholesterol | | " | May & Baker |

## 3.7 SAMPLE PREPARATION

### 3.7.1 Standard Metals

Stock standard solutions were prepared according to the recommendation of the Association of Analytical Chemist (AOAC, 1990) officials. Respective amount of metal salts were dissolved in 100cm$^3$ of de-ionised water to prepare 1.0 ppm solution (Appendix IV).

### 3.7.2 Blood Digestion

Each blood sample was treated with 2cm$^3$of concentrated nitric acid in a boiling tube heated to reflux on an electric hot plate at 70 – 90$^o$C for about 6 minutes. The content of the tube was allowed to cool, filtered through Whatman No. 42 filter paper into 50cm$^3$volumetric flask and made up to volume with de-ionized water. The flasks were then covered and kept for analysis (Anjorin *et al.,* 2010).

## 3.8 METAL ANALYSIS

The metals were analysed using the American society for testing materials (ASTM, 2002) method.

### 3.8.1 Atomic Absorption Spectrometry (AAS)

It is based on Desolvation – Vaporization – Atomization – Electronic Excitation. When a solution of analyte is aspirated into AAS, it mixes with gas to form aerosol which provides enough energy to vaporize analyte into molecular gas that dissociate into free atoms. The ground state atom absorbed radiation at

characteristic wavelength which pass through a monochromator and strike on the detector system. The decrease in intensity is directly proportional to the concentration of the sample analyte.

### 3.8.2 Instrumentation

Metal concentrations were determined on a Buck Scientific Model 210VGP atomic absorption spectrometer (AAS) equipped with a continuous background source connection. The instrument was set to zero using reagent blank. Each filtered acidified sample and standard was aspirated and its absorbance recorded. The result of each sample was the average of three (3) replicate readings. A calibration curve of absorbance against concentration of each element under investigation was constructed and finally the concentration of each element was determined from the calibration curve of its standard by interpolation.

## 3.9 BIOCHEMICAL TESTS

### 3.9.1 Haemoglobin Determination (Monica, 2003)

**Principle:**

Haemoglobin (cyanmethamoglobin method), whole blood is diluted in a buffered solution of potassium ferricyanide and potassium cyanide (Drabkin's solution). The red cells are haemolysed and haemoglobin is oxidized to methaemoglobin. This is converted by the cyanide to stable haemoglobincyanide (HiCN). The absorbance of the HiCN solution is read in a spectrophotometer at wavelength of 540nm (Monica, 2003).

**Procedure:**

Whole blood (20µl) sample was diluted in 5.0cm$^3$ of Drabkin's solution and was allowed to stand for 4 minutes. The test sample was read against the reagent blank using a spectrophotometer. The standard was also read in the same way, haemoglobin concentration of the unknown was determined when the absorbance of the sample ($A_{sample}$) was read against that of a standard ($A_{standard}$).

### 3.9.2 Glucose Determination (Trinder, 1969).

**Principle**

Glucose oxidase (GOD) catalyses the oxidation of glucose to give hydrogen peroxide ($H_2O_2$) and gluconic acid. In the presence of enzyme peroxidase, the hydrogen peroxide is broken down and the oxygen released reacts with 4-aminophenazone (4-aminoantipyrine) and phenol to give a pink colored complex. The intensity of the color is proportional to the concentration of serum glucose.

**Equation**

Glucose + Oxygen → Gluconic acid + $H_2O_2$

$H_2O_2$ +4-aminophenazone + phenol →Colored complex

**Procedure**

Test tubes were arranged and labeled in the following order: Test, Standard and Blank for all the samples. Phenol reagent (1.25 cm$^3$) were carefully pipetted into all the test tubes, followed by 0.025 cm$^3$ of serum into "Test" labeled tubes and

0.025 $cm^3$ distilled water to "Blank" labeled tube. Color reagent (1.25 cm3) was then added to all the test tubes, mixed and incubated for 20 minutes at 37 $^oC$. The absorbance was read at 570 nm using the "Blank" to zero the machine.

**Calculation**

Serum glucose concentration was calculated as follows;

$$\text{Glucose mmol/l} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 10$$

The glucose (mg) is converted to mmol/l as mg %

Glucose × 0.055 mmol/l

Normal Range = 3.89-5.84 (mmol/l)

### 3.9.3 Total Cholesterol Determination (Zlatkis *et al*., 1953)

**Principle**

Cholesterol extracted forms a red coloration with a mixture of ferric chloride acetic acid and sulfuric acid.

**Procedure**

Serum (0.05 $cm^3$) was pipetted using a micro pipette and transferred into a centrifuge tube. Ferric chloride acetic acid reagent (2.45 $cm^3$) was pipetted using a pressure pipette and added to the serum already in the centrifuge tube. The mixture was shaken for at least 10 seconds and allowed to stand for 10 minutes at room temperature. The mixture was then centrifuged for 5 minutes at a revolution

of 300 rpm to obtain a clear supernatant. Supernatant (1 $cm^3$) was then pipetted and transferred to a new test tube labeled unknown. To another test tube labeled standard, cholesterol working standard (1 $cm^3$) was pipetted and transferred. Ferric chloride acetic acid (1 $cm^3$) was then pipetted and transferred to another test tube labeled Blank.

Concentrated sulfuric acid was added carefully to each test tube and mixed thoroughly and allowed to stand for 25 minutes in the dark. The absorbance of the unknown and the standard solution was read against the blank in a colorimeter at a wave length of 560nm.

The absorbance of the unknown was taken as $A_u$ and that of the standard as $A_s$.

**Calculation**

The concentration of the total cholesterol in mg/dl was determined using;

$$\text{Total cholesterol (mg/dl)} = \frac{A_u \times Cs}{A_s}$$

Where

$A_u$ = Absorbance of the unknown/serum

$A_s$ = Absorbance of the standard

$C_s$ = Concentration of the standard

### 3.10 STATISTICAL ANALYSIS

The results of the four groups under study were compared statistically at ($P<0.05$) using Anova.

# CHAPTER FOUR

# RESULTS AND DISCUSSION

## 4.1 RESULTS

A total of 60 pregnant women, 20 each from first, second and third trimester and 20 non pregnant women attending antenatal and outpatient department of Kiru Comprehensive Health Center participated in this research. Mean age was found to be 24 years for pregnant women in their 1st trimester, 21 years for those in the 2nd trimester, 22 years for those in the 3rd trimester and 25 years for non pregnant women control. In the whole study group, the age ranges from 17 to 34 years, their height ranges from 1.45 to 1.80 meters, their weight ranges from 36 to 63 kilograms. The body mass index (BMI) ranges from 15 to 25 $kg/m^2$ and gestational age for the pregnant women range from 3 to 9 months (Appendices VI-IX).

Table 2 shows the distribution according to BMI range classification. It was found that in the pregnant women categories 16 (1st trimester), 14 (2nd trimester), 17 (3rd trimester) and 6 non pregnant women are in the normal BMI range class. In the underweight class, for pregnant women, 3 (1st trimester), 5 (2nd trimester) and 12 non pregnant women with none (0) in pregnant women (3rd trimester) fell under grade 1 underweight, with only 1 pregnant women each in the 1st and 3rd trimesters, that fell under grade 2 underweight and none (0) from other subjects. Equally, only 1 pregnant woman each from 2nd and 3rd trimesters fell in grade 3 underweight with none (0) from other subjects. In the overweight class, 1 pregnant woman (3rd trimester) and 2 non pregnant women were in

the grade 1 overweight with none (0) from other subjects and none was recorded for both grade 2 and grade 3 overweight.

Table 3 shows the distribution of BMI, Haemoglobin, Blood Pressure, Fasting Blood Glucose and Total Cholesterol among the subjects understudy. Pregnant women in the 3rd trimester group have higher value of mean BMI while the control group has the lowest. Highest mean value of Haemoglobin was obtained in the control group, on the other hand the values declined from the 1st trimester through the 3rd trimester. The mean BP value was found to be highest in the 3rd trimester and declined through the 2nd trimester, 1st trimester and the control group. Mean Fasting Blood Glucose was also found to be highest in the 3rd trimester group followed by 2nd trimester group and the 1st trimester with lowest mean value in the control group. Likewise, highest mean value of Total Cholesterol was obtained in the 3rd trimester group with lowest value in the 1st trimester.

Table 4 shows the distribution of the mineral elements analyzed. The highest mean values of the elements were obtained in the non pregnant women (control group). Similarly in the test group, pregnant women (1st trimester) had higher mean value which declined in the 2nd trimester through the 3rd trimester that had the lowest value.

Table 5 shows the distribution of BMI, Total Cholesterol and the eight mineral elements analyzed according to the sources of water consumed by the respondents. Highest mean BMI value was obtained in the group of respondents who consumed pipe borne water with lowest in the group utilizing water from pond/lake. Highest mean value of Total Cholesterol was obtained in the respondents consuming water from pond/lake with lowest value in the group consuming well water. Calcium, cobalt, copper and zinc have

highest mean values among the subjects utilizing water from pond/lake, iron and magnesium have highest values among the subjects consuming water from bore hole, while for chromium and lead highest values were obtained among subjects who utilize pipe borne water. Lowest mean values of calcium, cobalt, copper and zinc were obtained among subjects consuming well, bore hole, bore hole and well waters respectively, while the lowest mean values of iron, magnesium, chromium and lead were found among the subjects utilizing pipe borne, well water, well water and pond/lake in that order, as their sources of water for drinking and other domestic uses.

### 4.1.1 Percentage distribution of the educational levels of the households head and the respondents

Figure 1 presents percentage distribution of the educational levels of the households head and the respondents. The finding from this study revealed that 34% of the husbands, parent or guardians to the respondents had secondary education, 25% were either illiterate or lack formal education, 21% had tertiary education while the remaining 20% had primary education. These findings also revealed that 45% of the respondents are either illiterate or lack formal education, 28% had primary education, and 21% had attained secondary level, with only 6% having tertiary education.

### 4.1.2 Percentage distribution of the occupation of the households head and the respondents

Figure 2 presents percentage distributions of the occupation of the households head and the respondents. The finding from this study revealed that 48% of the husbands, parent or

guardians to the respondents under study were farmers, 25% were skilled workers, and 18% were traders while the remaining 10% were unskilled workers. These findings also revealed that 48% of the respondents were unskilled workers, 41% were traders, and 9% were skilled workers while the remaining 2% were farmers.

### 4.1.3 Percentage Distribution of the primary source of water for the households

Figure 3 presents percentage distribution of the primary source of water for drinking and other domestic uses for the households. The findings from this study revealed that 35% of the households use well water, 30% use bore holes, 27% utilize pipe borne water, while the remaining 8% use either pond or lake as their source of water.

### 4.1.4 Percentage Distribution of Food Consumed for breakfast by the respondents within the last 24 hours

Figure 4 presents foods consumed for breakfast within the last 24 hours. 69% had breakfast of pap made from millet, 14% from guinea corn, 12% from maize and 5% consumed pap made from other sources. Equally, beans cake consumed for breakfast shows 62% of the respondents had beans cake as their breakfast, 19% had beans plus other condiments for breakfast 12% had breakfast of beans with dry fish, while 7% had breakfast of beans with egg. These findings also revealed that 62% had tea and milk together with bread, 25% had tea, milk plus other beverages, with 13% who had tea and bread only for breakfast. Similarly unconventional foods consumed for breakfast shows; 45% had *awara* (a local cheese made from soybeans), 24% had *dan-malele* (a local food made from maize), 21% had potato for breakfast with remaining 10% who had food

made from other sources for breakfast. With regards to those who had taken left-over food *(dumame)* for breakfast, this findings revealed that 55% had *dumamen tuwo* for breakfast, 29 had rice and 16% had *dumame* from other sources of food as their breakfast.

### 4.1.5 Percentage distribution of food consumed for lunch by the respondents within the last 24 hours

Figure 5 presents the percentage distribution of food consumed by the respondents during lunch. The findings of this study revealed that 33% had Jallof rice, 27 had food from other sources for their lunch, and 22% had rice with stew, while 18% had rice, stew plus piece of meat or fish as their lunch. Distribution of local foods consumed for lunch shows that; 31% had *dan-wake* 20% had *waina,* 14% had *moi-moi* and the remaining 21% had local foods made from other sources for lunch. Similarly 63% had gruel millet (*kunu*) made from termerine, 23% made from pottash and 14% made from ground nut.

### 4.1.6 Percentage Distribution of Food Consumed for Supper by the respondents within the last 24 hours

Figure 6 presents the percentage distribution of food consumed as supper. 44% had *tuwo* made from maize, 34% had *tuwo* made from guinea corn, and 15% had *tuwo* made from semovita while 7% had not taken anything as supper. Conventional soup taken along with *tuwo* shows that; 42% had beans, 26% had piece of meat or fish, 14% had groundnuts while the remaining 18% had other sources of ingredients along with soup. Distribution of rice, spaghetti, or macaroni consumed by the respondents for supper shows that; 33%

had rice with stew, 22% had taken the food with piece of meat or fish, 12% had taken jallof rice while 33% also had consumed food made from other sources during supper.

### 4.1.7 Percentage distribution of food/drinks consumed in between meals by the respondents with the last 24 hours.

Figure 7 shows the distribution of food/drinks consumed in between meals. 21% had *zobo* drinks, 14% had yoghurt, 13% had leafy vegetables, 11% had fruits, 10% had *fura da nono*, 8% had taken food/drinks made from other sources in between meals and 23% had not taken anything (food/drinks) as in between meals within the last 24 hours before the interview.

**Table 2: Distributions of subjects according to BMI Range Classification**

| BMI Range | PW 1st Trimester | PW 2nd Trimester | PW 3rd Trimester | Non Preg Women | Grade |
|---|---|---|---|---|---|
| < 16.00 | 0 | 1 | 1 | 0 | Grade 3 UW |
| 16.00-16.99 | 1 | 0 | 1 | 0 | Grade 2 UW |
| 17.00-18.49 | 3 | 5 | 0 | 12 | Grade 1 UW |
| 18.50-24.99 | 16 | 14 | 17 | 6 | Normal |
| 25.00-29.99 | 0 | 0 | 1 | 2 | Grade 1 OW |
| 30.00-39.99 | 0 | 0 | 0 | 0 | Grade 2 OW |
| ≥ 40 | 0 | 0 | 0 | 0 | Grade 3 OW |

BMI = Body Mass Index

UW = Underweight

OW = Overweight

**Table 3: Mean BMI, Fasting Blood Glucose, Blood Pressure, Total Cholesterol and Haemoglobin levels in Pregnant and non pregnant women**

| Pregnant Women | BMI ($kg/m^{2)}$ | Glucose (mmol/l) | BP (mmHg) | TC (mg/dl) | Hb (mg/dl) |
|---|---|---|---|---|---|
| 1st Trimester (N=20) | 20.73±2.24 | 5.95±3.40 | 103.00±9.65/64.00±11.20 | 155.7±21.20 | 11.29±1.08 |
| 2nd Trimester (N=20) | 20.33±2.18 | 6.96±1.20 | 104.50±10.99/60.50±10.37 | 171.7±25.90 | 11.04±0.89 |
| 3rd Trimester (N=20) | 21.82±2.59 | 7.80±2.40 | 106.00±10.83/64.25±11.62 | 173.8±15.00 | 10.78±0.81 |
| Non Pregnant Women (N=20) | 19.38±2.36 | 5.30±2.30 | 102.50±11.10/63.50±14.70 | 166.9±20.50 | 11.38±1.24 |

Results are presented as Mean ± Standard Deviations (SD)

N = Number of subjects

BMI = Body Mass Index

BP = Blood Pressure

TC = Total cholesterol, Hb = Haemoglobin

**Table 4: Mean Elemental Levels in Pregnant and non pregnant women**

| Pregnant women | Calcium (Mg/dl) | Cobalt (Mg/dl) | Chromium (Mg/dl) | Copper (Mg/dl) | Iron (Mg/dl) | Magnesium (Mg/dl) | Zinc (Mg/dl) |
|---|---|---|---|---|---|---|---|
| 1st Trimester (N=20) | 2.495± 0.534 | 0.059± 0.048 | 0.060± 0.050 | 0.007± 0.005 | 0.515± 0.135 | 0.250± 0.100 | 0.275± 0.079 |
| 2nd Trimester (N=20) | 2.430± 0.485 | 0.056± 0.046 | 0.055± 0.050 | 0.005± 0.004 | 0.320± 0.089 | 0.195± 0.076 | 0.195± 0.010 |
| 3rd Trimester (N=20) | 2.273± 0.720 | 0.055± 0.044 | 0.032 0.003 | 0.004± 0.003 | 0.235± 0.114 | 0.130± 0.066 | 0.150± 0.061 |
| Non Pregnant Women (N=20) | 2.640± 0.623 | 0.065± 0.055 | 0.076± 0.057 | 0.012± 0.005 | 0.530± 0.134 | 0.275± 0.107 | 0.460± 0182 |

Results are presented as Mean ± Standard Deviations (SD)

N = Number of subjects

**Table 5: Mean values of BMI, serum Total Cholesterol, and Elemental Levels based on different water sources consumed by the respondents**

| SOURCE OF WATER | BMI ($kg/m^2$) | TOTAL CHOLESTEROL (mg/dl) | Ca (mg/dl) | Co (mg/dl) | Cr (mg/dl) | Cu (mg/dl) | Fe (mg/dl) | Mg (mg/dl) | Zn (mg/dl) |
|---|---|---|---|---|---|---|---|---|---|
| POND/LAKE<br>N = 6 | 17.60± 1.84 | 181.10± 17.5 | 2.630± 0.84 | 0.067± 0.052 | 0.055± 0.050 | 0.008± 0.007 | 0.383± 0.223 | 0.233± 0.137 | 0.830± 0.23 |
| WELL WATER<br>N = 28 | 20.80± 2.68 | 162.10± 16.50 | 2.340± 0.690 | 0.056± 0.050 | 0.053± 0.049 | 0.0063± 0.006 | 0.410± 0.130 | 0.164± 0.095 | 0.230± 0.128 |
| BORE HOLE<br>N = 24 | 20.50± 1.90 | 162.6± 22.7 | 2.470± 0.474 | 0.052± 0.050 | 0.052± 0.050 | 0.006± 0.005 | 0.413± 0.210 | 0.250± 1.10 | 0.296± 0.165 |
| PIPE BORNE<br>N = 22 | 21.00± 2.80 | 170.50± 23.80 | 2.560± 0.550 | 0.045± 0.031 | 0.055± 0.051 | 0.007± 0.006 | 0.380± 0.170 | 2.23± 0.80 | 0.260± 0.176 |

Results are presented as Mean ± Standard Deviations (SD)

N = Number of subjects

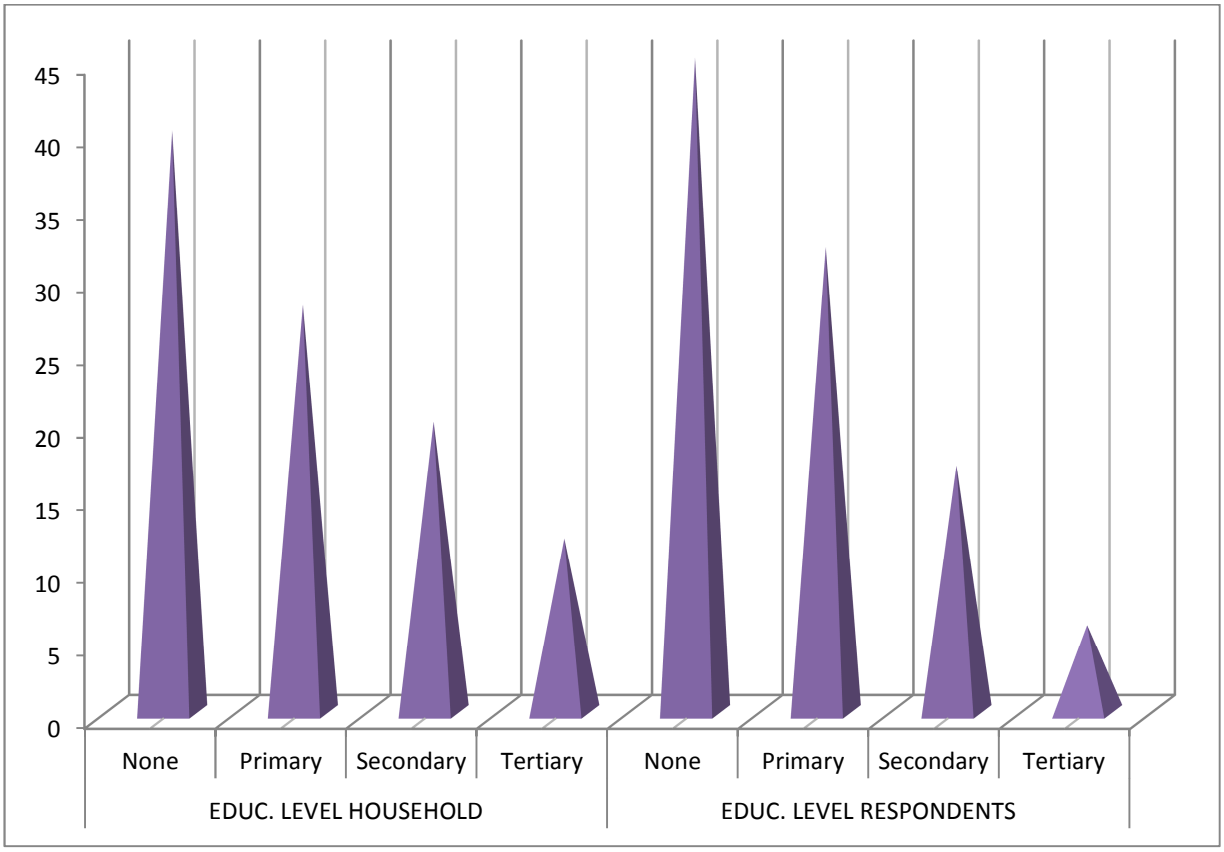


**Figure 1:** Percentage Distribution of the Educational Levels of Household heads and Respondents.

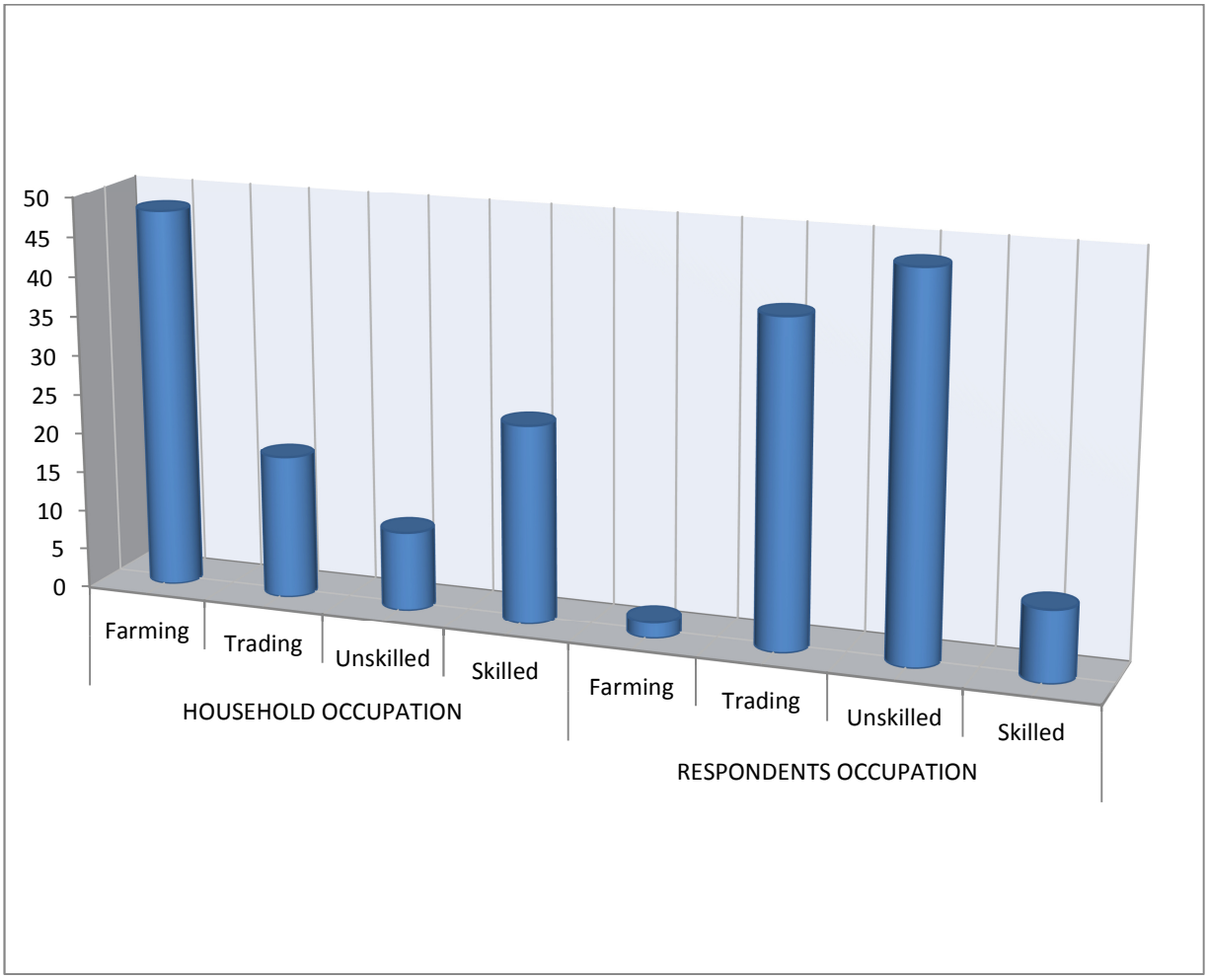


**Figure 2:** Percentage Distribution of the Household Heads and Respondents' Occupation

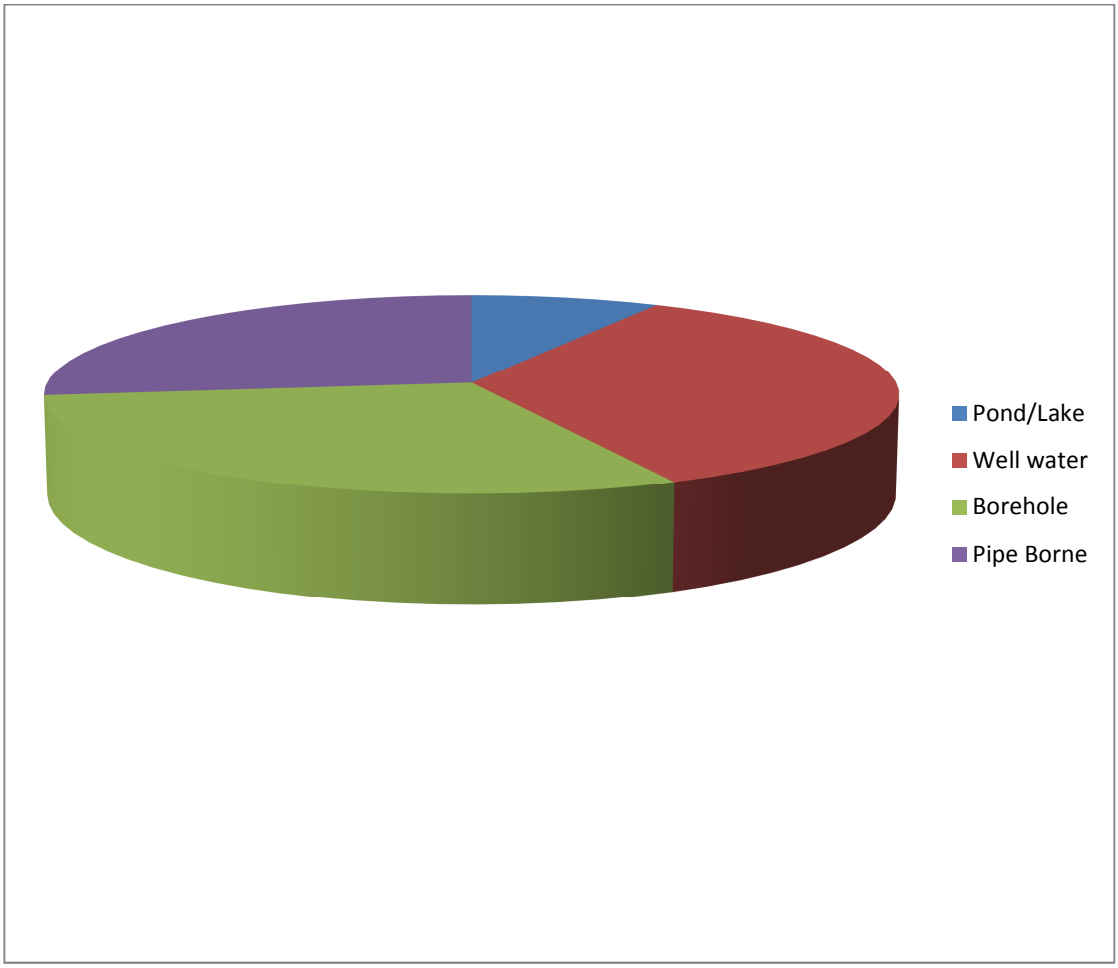


**Figure 3:** Percentage distribution of primary source of water for the households

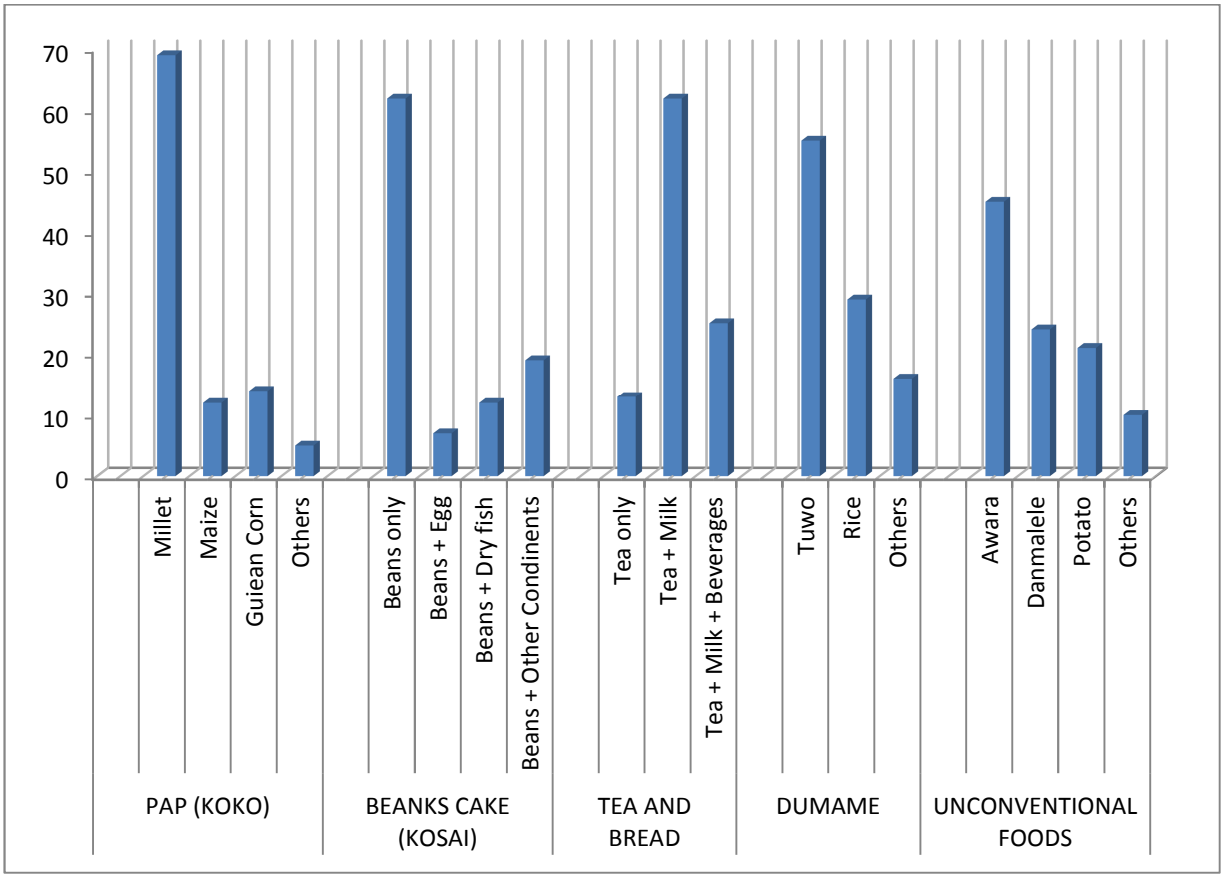


**Figure 4:** Percentage distribution of the food consumed for breakfast by participants within the last 24-hours before interview

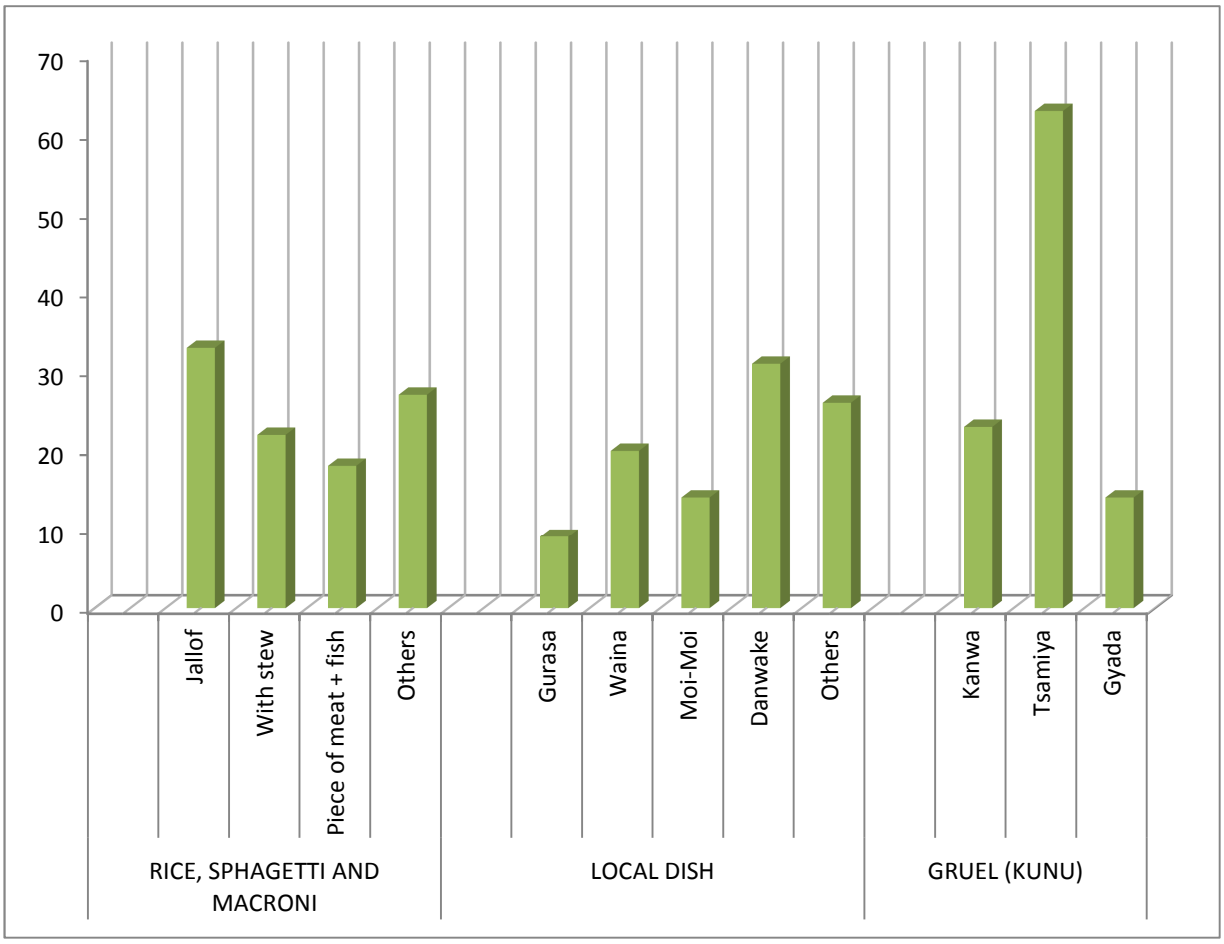


**Figure 5:** Percentage distribution of the food consumed for lunch by participants within the last 24-hours before interview

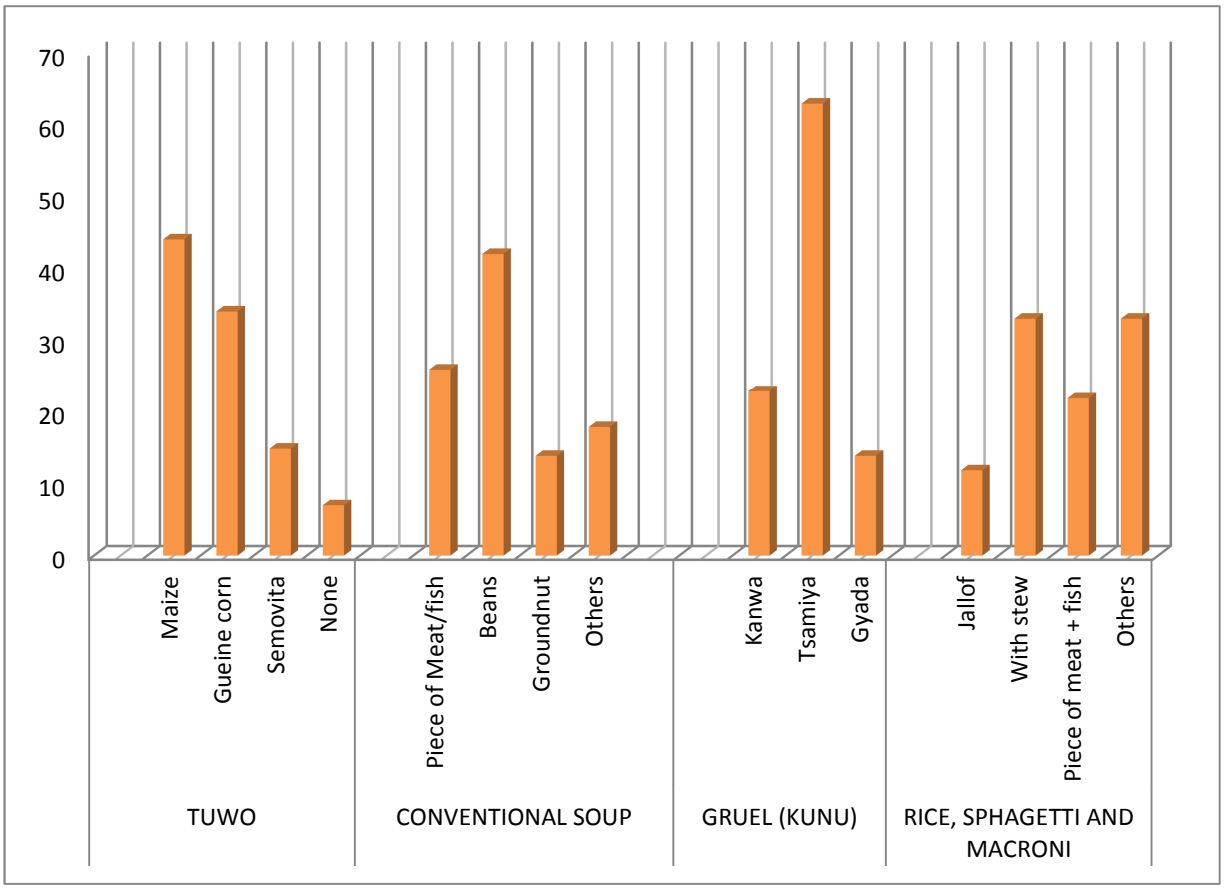


**Figure 6:** Percentage distribution of the food consumed for supper by participants within the last 24-hours before interview

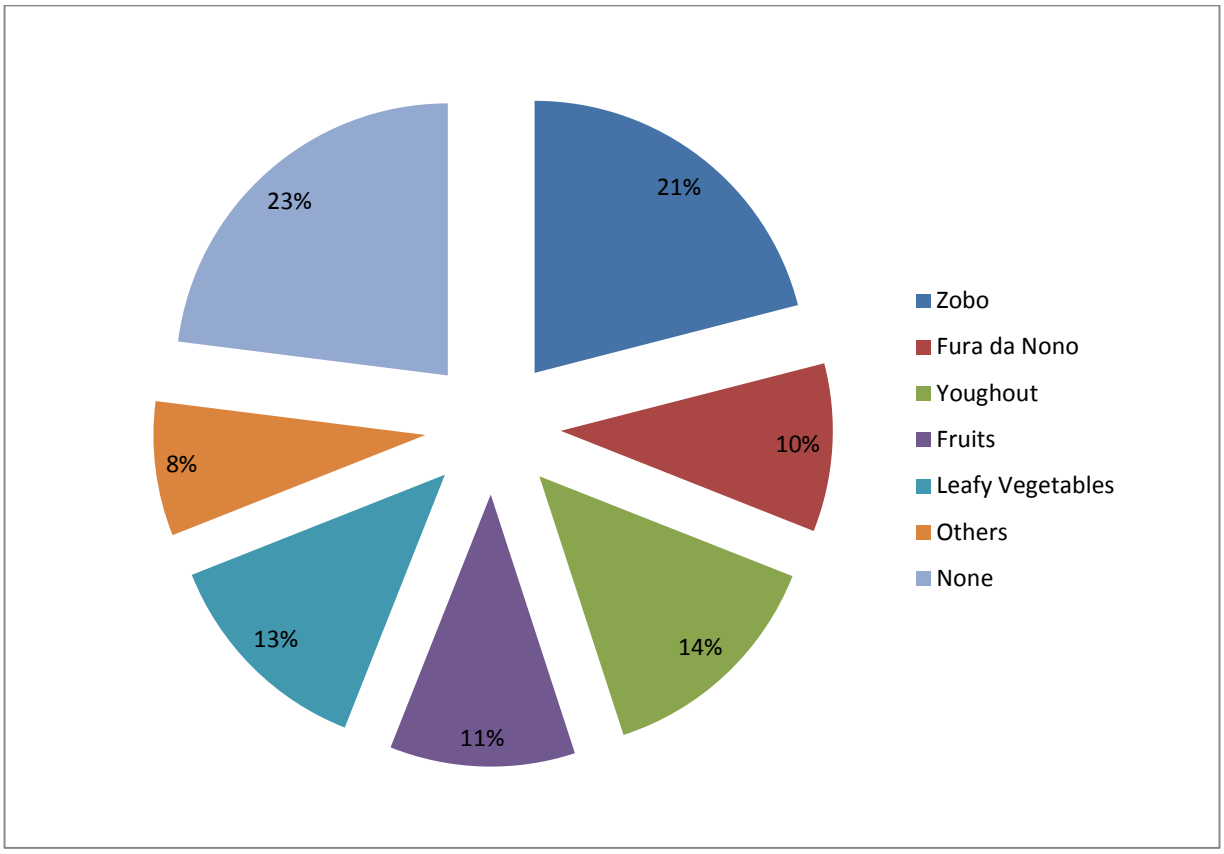


**Figure 7:** Percentage distribution of the food consumed in between meals by participants within the last 24-hours before interview

## 4.2 DISCUSSION

Figures 1-7 present the percentage distributions of the socio-economic status of the respondents and their household (education, primary sources of water for drinking and other domestic uses, sources of electricity). The results obtained from this study shows that there exists a progressive decline in the percentage of literate household heads and respondents, as the educational levels rose, which may therefore be a contributing factor to the prevalence of low nutrients status of both pregnant and non pregnant women observed in this study. WHO (2005) also reported that girls from less educated family (particularly young mothers) were more likely to be thin and short for their age and to have diets of poorer nutritional quality.

Likewise, the results obtained from the 24-hour dietary recall revealed that, the most available staple food that are major sources of energy (calories) were guinea corn, maize, millet, rice and flower, while the major sources of protein are soy beans and groundnuts. On the other hand, animal proteins were less consumed, so also fruits and vegetables were fairly consumed, apparently due to financial incapacity of the respondents/or household heads. Also from the results (Table 2) none of the subjects will be considered obese, since there exists strong correlation between obesity and socio-economic status as reported by Popkin *et al*. (1996).

The mean BMI values (Table 3) indicate that there is significant difference between the control group (non pregnant women), that has the lowest value compared to the test group (pregnant women). So, the result is in line with Law and Shiell (1996)

reports that the mean BMI value of the test group was significantly different from that of the control group ($p<0.05$).

The results also show that the mean haemoglobin level of the control group is higher when compared to that of the test group (Table 3). Because the iron required during pregnancy (3-4mg/dl) is substantial, risk of iron deficiency and iron deficiency anaemia should increase with gestation. According to Theresa (2005), the prevalence of anaemia and iron deficiency aneamia in the United States is substantial, particularly among the poor, and that during pregnancy, anaemia increased 4-folds from the 1st to the 3rd trimester in the low income women. In Nigeria, Brabin *et al*. (1997) found that adolescent girls who had low Hb (<10g/dl) were more likely to have a low BMI than those who had higher Hb levels, suggesting that overall nutrition is associated with anaemia. Although the maternal red cell mass and plasma volume both increase during gestation, they do not do so simultaneously. Haemoglobin and Hematocrit decline throughout the 1st and 2nd trimester reaching their lowest point in the 2nd to early in the 3rd trimester and then rise again nearer to term.

From Table 3 it can be seen that lowest mean systolic BP was obtained in the control group, while lowest mean diastolic BP was obtained in the test group (2nd trimester). However generally, there was significant difference between systolic and diastolic BP of the test and the control group ($p<0.05$), which is in line with what was reported by Law and Shiell (1996) that there was a significant difference between both the systolic and diastolic BP of the test and the control group.

In the pregnant women groups, the Fasting Blood Sugar level increases in the 1st and 2nd trimester with significant rise in the 3rd trimester (table 3), which disagrees with the report of Martin (1991) that FBS levels decreased as the pregnancy advanced. This could be attributed to the fact that number of parity they had, put them in the risk of developing gestational diabetes (Mann *et al*., 1983).

Total Cholesterol levels increase as the term approaches (Table 3). This is in line with the report of Passmore and Robinson (1974) that increasing lipolysis is proportional to the insulin deficiency.

The study also assessed the levels of seven elements in the 1st, 2nd and 3rd trimester of pregnancy and in non pregnant women. Significance difference ($p<0.05$) determined (Table 4) between the minerals values in the test and the control group confirmed the altered metabolism of some of these elements in pregnancy (Chaturvedi *et al*., 1996). The results also showed continued significant decline as the pregnancy progressed. This is in agreement with the study of (Law and Shiell (1996), who reported that mean serum concentration of the elements were significantly lower in the test group compared to the control. There is large body of evidence supporting the concept that deficiencies of micronutrients adversely affect maternal health and pregnancy outcome.

The mean calcium values of all the groups under study appeared to be within the normal range of values; because there is a little evidence that calcium deficiency is as much of an issue in non-Caucasian population of developing countries, other than in very limited areas as reported by Golden and Hambraeus, (1997). They suggested that

dietary intake may be higher in calcium than is usually assumed in the developing world, owing to the common practice of eating poultry, small animals, fish bones and of geophagia. The calcium values were comparable to literature ranges (Bucher *et al*., 2010). However it could be suggested that the seemingly normal serum calcium levels might not necessarily be interpreted as an outcome of adequate calcium intake, but rather as a results of calcium metabolism during periods of low intake.

Significance difference was obtained between magnesium levels in non pregnant and non pregnant women ($p<0.05$) (Table 4). This difference was obtained due to the increased need of this element during pregnancy and lactation, as reported by Bucher *et al*. (2010). The differences could be attributed to the type of dietary intake. Major dietary sources are whole grains, nuts, seeds, cocoa and green leafy plants. When compared to other published work by Bucher *et al*. (2010), the magnesium level determined in this work was higher. This could be due to the consumption of minimally processed food stuff, which was reported to contain low levels of magnesium and consequently give rise to low serum level of the element.

The same trend was also observed in case of zinc levels as seen in Table 4, the control group has the highest mean value, while in the test group the values decreased with increasing gestation. This is in agreement with the study of Shelia *et al*. (1981), who reported a continuous decrease in zinc concentration until term during pregnancy. Another study Henkin *et al*. (1971) has indicated a fall in the levels of total zinc, but similar values of free zinc in pregnant and non pregnant state.

The mean values of cobalt, chromium and copper were also significantly lower in the pregnant groups compared to the control group; it also shows a continuous significant decline with increasing duration of pregnancy.

# CHAPTER FIVE

# SUMMARY, CONCLUSION AND RECOMMENDATIONS

## 5.1 SUMMARY

The study was aimed at determining body weight and height (BMI kg/$m^2$), blood pressure, serum levels of Hb, fasting blood glucose, total cholesterol, calcium, cobalt, chromium, copper, iron, magnesium, and zinc in pregnant women (in the three trimesters) or test group and in non pregnant women that represent the control group. With specific objectives as; assessing the nutritional status of pregnant women using anthropometry and 24-hour dietary recall, identifying the statistical relationship between socio-economic data and determining the levels of BP, FBS, total cholesterol, some minerals elements as well as the correlations between these variables in the test and the control group.

A total of 80 apparently healthy women, attending ante-natal clinic and out-patient-department of Kiru Comprehensive Health Center, in Kiru Local Government Area of Kano State participated in the study, out of which 60 were pregnant women and 20 were non pregnant women control group. Fasting venous blood sample (5ml) was collected from each participant, using plastic disposable syringes. Each sample was divided into two, one portion was transferred into a sterilized lithium heparin container, carefully mixed and refrigerated in the laboratory, while serum was separated from the other portion and transferred into appropriately labeled sample bottles. The participants were also interviewed for anthropometrical variables (weight and height) that were measured according to standard techniques and calibrated instruments, while the biochemical tests

were conducted as follows; metals concentrations, haemoglobin, glucose, total cholesterol were determined using Atomic Absorption Spectrophotometry, Atomic Absorption spectrophotometry, Monica, (2003), Trinder, (1963) and Zlatkid et al., (1953) methods respectively.

The study revealed that there exist a progressive decline in the percentage of literate household heads and respondents, as the educational levels rose. Likewise according to BMI ranges none of the subjects will be considered obese, as there exists strong correlation between obesity and socio-economic status. More so, there is no significant difference ($p>0.05$) for BMI and Hb values, between the test and the control group. However the mean values of fasting blood glucose, systolic and diastolic BP, total cholesterol are significantly higher in the test when compared to the test group, while the mean values of the mineral elements, are significantly lower in the test when compared to the test group. Likewise the mean values of the mineral elements declined as the pregnancy progressed, this confirmed the altered metabolism of some of these elements during pregnancy.

## 5.2 CONCLUSION

The study revealed that pregnant women (especially those in the 3$^{rd}$ trimester) have higher BMI compared to the control group. However, all the values appeared normal, hence stunting BMI<16 or obesity BMI>30 were not recorded. Blood pressure, fasting blood glucose and total cholesterol appear to increase with advancing pregnancy, which suggests the risk of gestational hypertension and diabetes.

Moreover, the analytical results of serum calcium, cobalt, chromium, copper, iron, magnesium, and zinc, indicate the risk of deficiencies of these elements as the pregnancy progresses.

## 5.3 RECOMMEDATIONS

It is recommended that a base-line study of this nature should be undertaken in both urban and rural areas, in order to guard against mineral deficiency/over-load during pregnancy.

Since deficiencies of these nutrient elements adversely affect maternal health and birth outcome and not only one micronutrient alone is responsible for these effects, it is also recommended that the use of multivitamin supplementation to pregnant women during antenatal visits to health centers especially in rural areas should be encourage. This will improve maternal health and birth outcome and reduce the cases of pre-eclampsia and subsequent death cause by the deficiencies of some of these elements (most importantly calcium and magnesium).

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## Appendix I

### Bayero University Kano

### Faculty of Science, Department of Biochemistry (Research Questionnaire)

This questionnaire is designed to source information from pregnant women for use as part of dissertation in Masters of Science (M.Sc. Biochemistry), titled: "Anthropometry, Fasting Blood Glucose, Total Cholesterol and elemental profile in Pregnant women attending Kiru Comprehensive Health Center Kano State". All information obtained would be solely and exclusively used for research purposes.

Section A: Socio-Demographic Survey (Tick as appropriate).

1. How old are you?

i. 15-20yrs ii. 21-25yrs iii. 26-30yrs iv. 30 and above

2. What is your marital status?

i. Married ii. Divorced iii. Widowed iv. Others specify-

3. With whom do you stay?

i. Parent ii. Husband iii. Guardian iv. Alone

4. What is the highest qualification of your household head?

i. None ii. Primary ii. Secondary iv. Tertiary v. Others specify-

5. What is your highest qualification?

i. None ii. Primary iii. Secondary iv. Tertiary v. Others specify-

6. What is the primary occupation of your household head?

i. Farming ii. Trading iii. Unskilled worker iv. Skilled worker

7. What is your primary occupation?

i. Farming ii. Trading iii. Unskilled worker iv. Skilled worker

8. What is the source of water you use in your household?

i. Pond/Lake ii. Ground water iii. Borehole iv. Pipe borne v. Others specify

9. What is the electricity source of your household?

i. None ii. Standby generator iii. National electricity iv. Others Specify-

Section B: Assessment of Nutritional Status (Twenty four-hour dietary recall)

Now I will like you to tell me everything you have to eat and drink after you woke up yesterday morning. Including everything you ate and drank at home and away, even snacks, tea or coffee.

10. What do you eat during breakfast?

A. Pap (koko) made of:

i. Millet ii. Maize iii. Guinea-corn iv. Others specify_

B. Bean cake (kosai) made of:

i. Beans only ii. Beans and eggs iii. Beans and dried fish iv. Beans and other condiments

C. Tea with:

i. Tea only ii. Tea and milk iii. Tea, milk and beverages

D. Bread loaf size:

i. Small ii. Medium iii. Large

E. Left-over food (Dumame):

i. Tuwo ii. Rice iii. Others specify_

F. Unconventional food:

i. Awara ii. Dan-malele iii. Potato iv. Others specify_

11. What do you eat at lunch time?

A. Rice, spaghetti and / or macaroni:

i. Jallof only ii. With stew iii. Piece of meat/ or fish iv. Others specify_

B. Local dish:

i. Gurasa ii. Waina iii. Moi-moi iv. Dan-wake v. Others specify_

C. Gruel (kunu) type:

i. Kanwa ii. Tsamiya iii. Gyada

12. What do you eat during supper yesterday?

A. Tuwo type:

i. Maize ii. Millet iii. Guinea-corn iv. Semovita

B. Tuwo with:

i. Piece of meat and/ or fish ii. Beans iii. Ground nut iv. Others specify

D. Rice, spaghetti and / or macaroni:

i. Jallof only ii. With stew iii. Piece of meat/ or fish iv. Others specify_

13. Do you eat any food in between meal? If yes specify:

i. Zobo ii. Fura with Nono iii. Yoghurt iv. Fruit v. Leafy vegetables vi. Others specify_

14. Was the food intake usual? (Y/N)

15. Prove for sickness (Y/N): if yes, did the sickness affect appetite? (Y/N)

16. Probe for supplements (iron, anti-malaria, vitamins, and other supplements)? (Y/N), if yes specify

## Appendix II

**Bayero University, Kano**

**Department of Biochemistry**

**Consent Form**

This is to notify and seek your consent about a Master of Science degree research titled: "Anthropometry, Fasting Blood Glucose, Total Cholesterol and Elemental Profile in Pregnant Women attending Kiru Comprehensive Health Center Kano State**"** currently going on. This research work is solely for academic purpose and the results will be made available to you on request. Should you agree to cooperate to the research work? Please fill (1) and (2) below:

**1. Name of the respondent**

**2. Signature & Date/Thumb print of the respondent:**

Th**ank you for your anticipated cooperation.**

Kabiru Tukur

**(SPS/SCI/08/00848)**

## APPENDIX III

**Standard stock metals preparation for AAS Analysis (AOAC, 1990)**

**Standard calcium solution 1000ppm (1000µg/ml).**

A stock solution of calcium was prepared by dissolving 2.4973g of dry $CaCO_3$ in 10ml 50% HCl. The solution was warmed up to expel $CO_2$ and the volume was made to 1 liter with de-ionized water. Series of calcium standard solutions with concentration range 0 – 10ppm were prepared by appropriate dilution of the stock solution.

**Standard Chromium Solution 1000ppm (1000µg/ml)**

A stock solution of chromium was prepared by dissolving 28.28g of $K_2Cr_2O_7$ of distilled water and diluted to 1 liter with de-ionized water. Series of chromium standard solutions with concentration range 0 – 10ppm were prepared by appropriate dilution of the stock solution.

**Standard Cobalt Solution 1000ppm (1000µg/ml)**

A stock solution of cobalt was prepared by dissolving 4.037g of $CoCl_2.6H_2O$ in 10ml distilled water and diluted to 1 liter with de-ionized water. Series of cobalt standard solutions with concentration range 0 – 10ppm were prepared by appropriate dilution of the stock solution.

**Standard Copper Solution 1000ppm (1000µg/ml)**

A stock solution of copper was prepared by dissolving 1g of electric copper in 15ml of 50% nitric acid followed by the addition of 4ml of $H_2SO_4$ and heat. It was finally diluted

with de-ionized water to 1 liter. Series of copper standard solutions with concentration range 0 – 1 ppm were prepared by appropriate dilution of the stock solution.

**Standard Iron Solution 1000 ppm (1000µg/ml)**

A stock solution of iron was prepared by dissolving 4.977g of $FeSO_4.7H_2O$ in 10ml 50% Nitric acid and finally diluted to 1 liter with de-ionized water. Series of iron standard solutions with concentration range 0 – 10ppm were prepared by appropriate dilution of the stock solution.

**Standard Zinc Solution 1000ppm (1000µg/ml).**

A stock solution of Zinc was prepared by dissolving 4.399g of $ZnSO_4.7H_2O$ in 10ml 50% nitric acid and diluted to 1 liter with de-ionized water. Series of Zinc standard solutions with concentration range of 0 – 10ppm was prepared by appropriate dilution of the stock solution.

## APPENDIX IV

## PREPARATION OF REAGENTS

### Glucose Determination (Trinder, 1969).

The reagents used for the determination of glucose levels are:

### Phenol Reagents

Sodium chloride (9g) was weighted and dissolved in 100 $cm^3$ volumetric flask by addition of 1000 $cm^3$ distilled water. Then 1.94g of weighted phenol crystals was dissolved in 1000 $cm^3$ of the Sodium Chloride solution.

### Acetate Buffer

Sodium acetate (12.58g) was dissolved in 1000 $cm^3$ distilled water. Then 0.38 $cm^3$ of glacial acetic acid was added.

### Color Reagents

4-aminophenazone (0.3g) was dissolved in a 1000 $cm^3$ acetate buffer and 15 $cm^3$ fermcozyme (glucose oxidase/peroxidase), solution was added.

### Glucose Standard

Glucose (100mg) was dissolved in 1 % benzoic acid (50 $cm^3$) and allowed to stand overnight before use. The reagents were transferred into separate labeled storage bottles and stored at 2-8 $^0C$.

**Total Cholesterol Determination (Zlatkis *et al*., 1953)**

**Preparation of Reagents**

The reagents used for the determination of total cholesterol levels are;

**Ferric Chloride Acetic Acid Reagents (0.7 w/v)**

Ferric chloride ($FeCl_3.6H_2O$) (0.7g) was weighted and dissolved in 100 $cm^3$ glacial acetic acid

**Cholesterol Stock Standard (0.1 % w/v)**

Cholesterol (0.1g) was measured and dissolved in 100 $cm^3$of pure glacial acetic acid

**Working Standard**

The stock standard was diluted to 1:25 with ferric chloride acetic acid reagents by taking 4 $cm^3$ of cholesterol standard using pipette and then transferred into 100 $cm^3$ glass cylinder. The stock standard was then diluted up to 100 $cm^3$ mark of the cylinder with ferric chloride acetic acid reagent and kept in a cool dark place.

**APPENDIX V (a) CONCENTRATION (ppm) PREGNANT WOMEN (FIRST TRIMESTER)**

| | Ca | Co | Cr | Cu | Fe | Mg | Zn |
|---|---|---|---|---|---|---|---|
| $F_1$ | 19.00 | - | 1.00 | 0.154 | 4.00 | 3.00 | 1.00 |
| $F_2$ | 28.50 | - | 1.00 | 0.077 | 7.00 | 2.00 | 3.00 |
| $F_3$ | 24.50 | 1.00 | 1.00 | - | 5.00 | 3.00 | 3.00 |
| $F_4$ | 26.50 | 1.00 | 1.00 | 0.077 | 5.00 | 3.00 | 4.00 |
| $F_5$ | 22.50 | 1.00 | 1.00 | 0.077 | 6.00 | 3.00 | 2.00 |
| $F_6$ | 20.00 | - | 1.00 | 0.077 | 7.00 | 2.00 | 2.00 |
| $F_7$ | 17.00 | - | - | - | 4.00 | 3.00 | 3.00 |
| $F_8$ | 16.00 | 1.00 | 1.00 | 0.077 | 4.00 | 3.00 | 3.00 |
| $F_9$ | 28.50 | 1.00 | 1.00 | - | 8.00 | 4.00 | 4.00 |
| $F_{10}$ | 20.50 | 1.00 | 1.00 | 0.077 | 7.00 | 4.00 | 3.00 |
| $F_{11}$ | 32.00 | - | - | 0.077 | 5.00 | 2.00 | 2.00 |
| $F_{12}$ | 20.50 | 1.00 | - | 0.077 | 6.00 | 1.00 | 2.00 |
| $F_{13}$ | 26.50 | 1.00 | 1.00 | 0.154 | 6.00 | 1.00 | 3.00 |
| $F_{14}$ | 24.50 | 1.00 | - | - | 4.00 | 1.00 | 3.00 |
| $F_{15}$ | 28.00 | - | - | 0.077 | 5.00 | 3.00 | 3.00 |
| $F_{16}$ | 26.50 | - | - | - | 4.00 | 2.00 | 4.00 |
| $F_{17}$ | 21.50 | - | - | - | 4.00 | 3.00 | 3.00 |
| $F_{18}$ | 27.50 | - | - | 0.077 | 3.00 | 2.00 | 3.00 |
| $F_{19}$ | 34.00 | - | 1.00 | - | 5.00 | 1.00 | 2.00 |
| $F_{20}$ | 35.00 | 1.00 | 1.00 | 0.077 | 4.00 | 4.00 | 1.00 |

**APPENDIX V (b) CONCENTRATION (ppm) PREGNANT WOMEN (SECOND TRIMESTER)**

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| $S_1$ | 17.50 | 1.00 | 1.00 | | 3.00 | 1.00 | 4.00 |
| $S_2$ | 24.00 | - | - | 0.077 | 3.00 | 2.00 | 4.00 |
| $S_3$ | 24.50 | - | - | - | 3.00 | 2.00 | 4.00 |
| $S_4$ | 26.50 | - | - | 0.077 | 3.00 | 2.00 | 2.00 |
| $S_5$ | 21.50 | - | 1.00 | 0.077 | 3.00 | 1.00 | 1.00 |
| $S_6$ | 21.00 | 1.00 | - | 0.077 | 3.00 | 3.00 | 2.00 |
| $S_7$ | 23.50 | - | - | 0.077 | 4.00 | 2.00 | 2.00 |
| $S_8$ | 31.50 | - | - | 0.077 | 2.00 | 1.00 | 2.00 |
| $S_9$ | 28.50 | 1.00 | 1.00 | 0.077 | 4.00 | 2.00 | 2.00 |
| $S_{10}$ | 20.00 | 1.00 | - | 0.077 | 3.00 | 1.00 | 1.00 |
| $S_{11}$ | 26.50 | 1.00 | - | 0.077 | 5.00 | 1.00 | 2.00 |
| $S_{12}$ | 30.50 | 1.00 | 1.00 | - | 2.00 | 1.00 | 1.00 |
| $S_{13}$ | 15.50 | 1.00 | 1.00 | 0.154 | 3.00 | 3.00 | 1.00 |
| $S_{14}$ | 19.50 | - | - | - | 2.00 | 3.00 | 1.00 |
| $S_{15}$ | 24.50 | - | 1.00 | - | 4.00 | 2.00 | 1.00 |
| $S_{16}$ | 34.50 | - | 1.00 | - | 5.00 | 2.00 | 2.00 |
| $S_{17}$ | 28.00 | 1.00 | - | - | 3.00 | 3.00 | 2.00 |
| $S_{18}$ | 20.50 | 1.00 | - | - | 4.00 | 2.00 | 1.00 |
| $S_{19}$ | 26.50 | - | 1.00 | - | 3.00 | 3.00 | 2.00 |
| $S_{20}$ | 21.50 | - | - | 0.077 | 2.00 | 2.00 | 2.00 |

**APPENDIX V(c) CONCENTRATION (ppm) PREGNANT WOMEN (THIRD TRIMESTER)**

| | Ca | Co | Cr | Cu | Fe | Mg | Zn |
|---|---|---|---|---|---|---|---|
| $T_1$ | 24.00 | - | 1.00 | 0.077 | 3.00 | 1.00 | 1.00 |
| $T_2$ | 34.00 | - | - | - | 3.00 | 2.00 | 1.00 |
| $T_3$ | 21.50 | - | - | 0.077 | 2.00 | 2.00 | 2.00 |
| $T_4$ | 30.00 | - | - | - | 4.00 | 1.00 | 1.00 |
| $T_5$ | 07.00 | 1.00 | - | 0.077 | 4.00 | 1.00 | 2.00 |
| $T_6$ | 23.00 | - | - | 0.077 | 3.00 | - | 1.00 |
| $T_7$ | 18.00 | - | - | - | 3.00 | - | - |
| $T_8$ | 08.50 | - | - | - | 3.00 | 1.00 | 2.00 |
| $T_9$ | 30.00 | - | - | 0.077 | 2.00 | 1.00 | 2.00 |
| $T_{10}$ | 21.50 | - | - | 0.077 | 3.00 | 2.00 | 2.00 |
| $T_{11}$ | 17.00 | - | 1.00 | 0.077 | 2.00 | 2.00 | 2.00 |
| $T_{12}$ | 23.50 | 1.00 | - | 0.077 | 3.00 | 2.00 | 1.00 |
| $T_{13}$ | 25.00 | 1.00 | - | 0.077 | 3.00 | 2.00 | 1.00 |
| $T_{14}$ | 29.50 | 1.00 | - | 0.077 | 1.00 | 2.00 | 1.00 |
| $T_{15}$ | 15.50 | 1.00 | - | - | 1.00 | 1.00 | 2.00 |
| $T_{16}$ | 17.50 | - | - | 0.077 | - | 1.00 | 2.00 |
| $T_{17}$ | 24.00 | 1.00 | - | - | - | 2.00 | 1.00 |
| $T_{18}$ | 26.00 | - | - | - | 3.00 | 1.00 | 2.00 |
| $T_{19}$ | 32.00 | - | - | - | 2.00 | 1.00 | 2.00 |
| $T_{20}$ | 26.50 | 1.00 | - | - | 2.00 | 1.00 | 2.00 |

**APPENDIX V (d) CONCENTRATION (ppm) NON PREGNANT WOMEN**

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| $N_1$ | 28.00 | 1.00 | 1.00 | 0.154 | 4.00 | 3.00 | 6.00 |
| $N_2$ | 31.50 | 1.00 | 1.00 | 0.154 | 6.00 | 3.00 | 5.00 |
| $N_3$ | 28.00 | 1.00 | 1.00 | 0.154 | 5.00 | 3.00 | 5.00 |
| $N_4$ | 42.50 | 1.00 | 1.00 | 0.076 | 4.00 | 4.00 | 7.00 |
| $N_5$ | 27.00 | 1.00 | - | 0.076 | 6.00 | 3.00 | 5.00 |
| $N_6$ | 30.50 | 1.00 | 1.00 | 0.076 | 4.00 | 4.00 | 4.00 |
| $N_7$ | 19.50 | - | 1.00 | 0.154 | 6.00 | 3.00 | 6.00 |
| $N_8$ | 16.50 | - | 1.00 | 0.154 | 6.00 | 5.00 | 9.00 |
| $N_9$ | 27.50 | - | - | 0.154 | 4.00 | 3.00 | 5.00 |
| $N_{10}$ | 15.50 | - | - | 0.231 | 7.00 | 4.00 | 4.00 |
| $N_{11}$ | 28.00 | - | - | 0.154 | 5.00 | 2.00 | 3.00 |
| $N_{12}$ | 35.00 | - | 2.00 | 0.076 | 5.00 | 2.00 | 4.00 |
| $N_{13}$ | 27.50 | - | - | 0.154 | 5.00 | 2.00 | 5.00 |
| $N_{14}$ | 22.50 | 1.00 | 1.00 | - | 7.00 | 2.00 | 6.00 |
| $N_{15}$ | 24.50 | - | - | 0.076 | 8.00 | 2.00 | 4.00 |
| $N_{16}$ | 30.50 | 1.00 | 1.00 | 0.154 | 7.00 | 3.00 | 4.00 |
| $N_{17}$ | 24.00 | 1.00 | 1.00 | 0.076 | 3.00 | 2.00 | 3.00 |
| $N_{18}$ | 21.00 | - | 1.00 | 0.154 | 6.00 | 3.00 | 3.00 |
| $N_{19}$ | 27.00 | 1.00 | 1.00 | 0.076 | 4.00 | 2.00 | - |
| $N_{20}$ | 22.00 | 1.00 | - | 0.154 | 4.00 | - | 4.00 |

## APPENDIX (VI)

## PREGNANT WOMEN (FIRST TRIMESTER)

| SAMPLE NO. | AGE (yrs) | B.P (mg/dl) | WEIGHT (kg) | HEIGHT (m) | BMI ($kg/M^{2)}$ | Hb (mg/dl) | GLUCOSE (mmol/l) | TOT. CHOL (mg/dl) |
|---|---|---|---|---|---|---|---|---|
| $F_1$ | 22 | 100/60 | 53 | 168 | 23.04 | 12.50 | - | - |
| $F_2$ | 26 | 95/50 | 61 | 161 | 23.46 | 11.00 | 5.50 | 164.40 |
| $F_3$ | 29 | 110/75 | 57 | 159 | 22.80 | 11.00 | 3.80 | 172.40 |
| $F_4$ | 34 | 100/60 | 51 | 152 | 22.20 | 10.90 | - | - |
| $F_5$ | 19 | 100/60 | 52 | 164 | 19.25 | 10.90 | - | - |
| $F_6$ | 18 | 110/80 | 55 | 164 | 20.40 | 9.00 | - | - |
| $F_7$ | 24 | 100/60 | 52 | 155 | 21.67 | 10.90 | - | - |
| $F_8$ | 20 | 100/70 | 50 | 152 | 21.74 | 11.00 | 2.50 | 120.00 |
| $F_9$ | 17 | 120/60 | 47 | 152 | 20.40 | 10.90 | - | - |
| $F_{10}$ | 32 | 120/60 | 54 | 160 | 20.80 | 12.50 | - | - |
| $F_{11}$ | 23 | 100/60 | 60 | 157 | 24.00 | 10.90 | 11.20 | 168.90 |
| $F_{12}$ | 19 | 120/90 | 51 | 156 | 22.92 | 12.50 | 6.68 | 152.80 |
| $F_{13}$ | 20 | 90/50 | 41 | 150 | 17.83 | 12.50 | - | - |
| $F_{14}$ | 22 | 100/55 | 56 | 160 | 21.54 | 11.00 | - | - |
| $F_{15}$ | 28 | 90/55 | 49 | 157 | 19.60 | 9.00 | - | - |
| $F_{16}$ | 33 | 100/70 | 53 | 158 | 21.20 | 12.50 | - | - |
| $F_{17}$ | 20 | 90/50 | 44 | 164 | 16.30 | 12.50 | - | - |
| $F_{18}$ | 25 | 100/80 | 45 | 160 | 17.30 | 11.00 | - | - |
| $F_{19}$ | 30 | 95/60 | 40 | 152 | 17.40 | 12.50 | - | - |
| $F_{20}$ | 18 | 110/75 | 55 | 161 | 21.20 | 10.90 | - | - |
| MEAN & S.D | 24.± 5.4 | 103/64± 9.7/±11.2 | 51.5± 5.76 | 158.1± 4.93 | 20.73± 2.2 | 11.29± 1.08 | 5.94± 3.40 | 155.71± 21.20 |

## APPENDIX (VII)

## PREGNANT WOMEN (SECOND TRIMESTER)

| | AGE (yrs) | B.P (mg/dl) | WEIGHT (kg) | HEIGHT (m) | BMI ($kg/M^{2)}$ | Hb (mg/dl) | GLUCOSE (mmol/l) | TOT. CHOL (mg/dl) |
|---|---|---|---|---|---|---|---|---|
| $S_1$ | 20 | 100/60 | 57 | 154 | 23.75 | 11.00 | 6.60 | 152.50 |
| $S_2$ | 24 | 110/75 | 43 | 152 | 18.70 | 10.50 | - | - |
| $S_3$ | 19 | 110/60 | 45 | 153 | 19.57 | 11.00 | - | - |
| $S_4$ | 20 | 130/60 | 56 | 166 | 20.00 | 10.90 | 6.73 | 154.90 |
| $S_5$ | 22 | 90/60 | 42 | 152 | 18.26 | 10.90 | - | - |
| $S_6$ | 19 | 100/50 | 50 | 180 | 15.63 | 12.50 | - | - |
| $S_7$ | 30 | 90/40 | 56 | 156 | 23.33 | 12.50 | - | - |
| $S_8$ | 20 | 110/75 | 56 | 159 | 22.40 | 11.00 | 10.90 | 187.40 |
| $S_9$ | 18 | 100/50 | 54 | 169 | 18.62 | 10.90 | - | - |
| $S_{10}$ | 23 | 90/50 | 54 | 154 | 22.50 | 12.00 | - | - |
| $S_{11}$ | 20 | 110/50 | 61 | 165 | 22.60 | 11.00 | - | - |
| $S_{12}$ | 18 | 100/70 | 55 | 168 | 19.60 | 11.00 | - | - |
| $S_{13}$ | 19 | 100/60 | 49 | 163 | 18.15 | 10.90 | - | - |
| $S_{14}$ | 19 | 120/70 | 51 | 159 | 20.40 | 10.00 | - | - |
| $S_{15}$ | 25 | 110/80 | 58 | 162 | 22.30 | 12.50 | 5.50 | 153.50 |
| $S_{16}$ | 20 | 120/70 | 44 | 156 | 18.33 | 8.50 | 10.30 | 210.00 |
| $S_{17}$ | 18 | 100/50 | 58 | 161 | 22.30 | 12.50 | - | - |
| $S_{18}$ | 24 | 90/60 | 47 | 161 | 18.07 | 11.00 | - | - |
| $S_{19}$ | 26 | 100/60 | 56 | 164 | 20.70 | 10.90 | - | - |
| $S_{20}$ | 32 | 110/60 | 49 | 151 | 21.30 | 10.90 | - | - |
| MEAN & S.D | 21.4±3.6 | 104.5/60.5 ±10.9/10.4 | 52.55± 6.31 | 162.25± 7.25 | 20.33± 2.81 | 11.04± 0.89 | 6.96± 1.20 | 171.7± 25.9 |

## APPENDIX (VIII)

## PREGNANT WOMEN (THIRD TRIMESTER)

| | AGE (yrs) | B.P (mg/dl) | WEIGHT (kg) | HEIGHT (m) | BMI ($kg/M^{2)}$ | Hb (mg/dl) | GLUCOSE (mmol/l) | TOT. CHOL (mg/dl) |
|---|---|---|---|---|---|---|---|---|
| $T_1$ | 23 | 110/75 | 62 | 157 | 24.80 | 12.00 | 5.80 | 198.70 |
| $T_2$ | 20 | 120/75 | 50 | 145 | 25.00 | 10.90 | - | - |
| $T_3$ | 18 | 95/60 | 52 | 174 | 17.33 | 11.00 | - | - |
| $T_4$ | 19 | 110/70 | 55 | 152 | 23.90 | 10.90 | 10.25 | 165.70 |
| $T_5$ | 19 | 90/50 | 51 | 156 | 21.25 | 10.90 | - | - |
| $T_6$ | 20 | 100/55 | 49 | 154 | 20.42 | 11.00 | - | - |
| $T_7$ | 18 | 100/65 | 52 | 158 | 20.80 | 11.00 | - | - |
| $T_8$ | 24 | 120/80 | 63 | 167 | 22.50 | 10.90 | 6.73 | 169.00 |
| $T_9$ | 20 | 110/70 | 53 | 162 | 23.00 | 11.00 | - | - |
| $T_{10}$ | 20 | 100/55 | 50 | 157 | 20.00 | 12.50 | - | - |
| $T_{11}$ | 17 | 100/90 | 54 | 159 | 22.08 | 10.90 | - | - |
| $T_{12}$ | 19 | 120/50 | 53 | 154 | 22.08 | 10.00 | 5.50 | 160.10 |
| $T_{13}$ | 25 | 110/80 | 62 | 159 | 24.80 | 10.90 | - | - |
| $T_{14}$ | 20 | 100/60 | 64 | 161 | 24.62 | 11.00 | - | - |
| $T_{15}$ | 18 | 100/60 | 58 | 157 | 23.20 | 11.00 | - | - |
| $T_{16}$ | 19 | 95/50 | 49 | 152 | 21.30 | 10.90 | - | - |
| $T_{17}$ | 28 | 90/55 | 36 | 156 | 15.00 | 10.00 | - | - |
| $T_{18}$ | 27 | 110/50 | 54 | 158 | 21.60 | 9.00 | - | - |
| $T_{19}$ | 20 | 130/70 | 59 | 158 | 23.60 | 10.90 | 10.50 | 175.50 |
| $T_{20}$ | 19 | 110/70 | 48 | 159 | 19.20 | 9.00 | - | - |
| MEAN & S.D | 20.7± 3.1O | 106/64.3± 64.25/11.62 | 53.7O± 6.53 | 157.8O± 5.38 | 21.82± 2.59 | 10.78± 0.81 | 7.80± 2.40 | 173.80± 15.00 |

## APPENDIX (IX)

## NON PREGNANT WOMEN (CONTROL)

| | AGE (yrs) | B.P (mg/dl) | WEIGHT (kg) | HEIGHT (m) | BMI ($kg/M^{2)}$ | Hb (mg/dl) | GLUCOSE (mmol/l) | TOT. CHOL (mg/dl) |
|---|---|---|---|---|---|---|---|---|
| $N_1$ | 25 | 90/40 | 42 | 150 | 18.26 | 10.90 | - | 165.5 |
| $N_2$ | 27 | 90/50 | 60 | 150 | 26.08 | 12.50 | 6.50 | 155.5 |
| $N_3$ | 29 | 110/75 | 41 | 153 | 17.82 | 12.50 | - | - |
| $N_4$ | 22 | 90/50 | 42 | 156 | 17.50 | 11.00 | - | 177.8 |
| $N_5$ | 20 | 90/50 | 45 | 158 | 18.00 | 9.00 | 3.00 | 150.7 |
| $N_6$ | 23 | 110/70 | 56 | 161 | 21.54 | 12.50 | 4.75 | 162.5 |
| $N_7$ | 31 | 130/90 | 55 | 160 | 20.40 | 12.50 | 4.50 | 144.8 |
| $N_8$ | 24 | 100/60 | 47 | 157 | 18.80 | 11.00 | - | - |
| $N_9$ | 30 | 90/60 | 50 | 152 | 21.70 | 12.50 | 3.50 | 202.6 |
| $N_{10}$ | 23 | 90/40 | 43 | 157 | 17.20 | 10.90 | - | - |
| $N_{11}$ | 20 | 130/90 | 44 | 152 | 19.00 | 9.00 | - | - |
| $N_{12}$ | 22 | 100/50 | 47 | 155 | 19.60 | 10.90 | 4.30 | 147.4 |
| $N_{13}$ | 29 | 100/70 | 43 | 159 | 17.20 | 12.50 | - | - |
| $N_{14}$ | 25 | 100/80 | 43 | 155 | 17.90 | 12.50 | 4.00 | - |
| $N_{15}$ | 32 | 110/60 | 41 | 156 | 17.0 | 11.00 | - | - |
| $N_{16}$ | 19 | 95/60 | 46 | 157 | 18.26 | 10.90 | - | - |
| $N_{17}$ | 24 | 100/65 | 58 | 159 | 26.08 | 12.50 | 4.50 | - |
| $N_{18}$ | 18 | 120/80 | 40 | 150 | 17.82 | 9.00 | - | - |
| $N_{19}$ | 20 | 100/70 | 47 | 153 | 17.50 | 12.00 | 7.36 | 162.3 |
| $N_{20}$ | 30 | 100/60 | 55 | 161 | 18.00 | 12.00 | 10.55 | 200.0 |
| MEAN & S.D | 24.70± 4.30 | 102.5/63.5± 12.41/14.70 | 47.25± 6.24 | 155.58± 3.61 | 19.38± 2.36 | 11.38± 1.24 | 5.30± 2.30 | 166.90± 20.50 |