CHAPTER ONE

INTRODUCTION

1.1 Background

Pregnancy is defined as the period from conception to birth. It is a state in which a woman carries a fertilized egg inside her body. It develops into the placenta and embryo, and later into a foetus. The foetus might be one or more than one developing inside the woman's uterus. Pregnancy may be either natural or assisted. Pregnancy or gestation usually lasts approximately 40 weeks, beginning from the first day of the woman's last menstrual period, and is customarily divided into three trimesters, each of which is slightly longer than 13 weeks (or lasting three months). By convention, the first trimester, 0 to 13 weeks, begins on the first day of the last menses. Conception period and some weeks thereafter are the days when most organs and systems of the foetus are formed. During the second trimester, 13 to 26 weeks, rapid fetal growth occurs and by the third trimester, 26 to 40 weeks, is the period in which fetal organs complete maturation (Ashwood, 2001). The energy needed to develop these systems comes from the energy and nutrients in the mother's circulation and metabolism; hence adequate micronutrient levels during pregnancy is particularly crucial (Ogbodo *et al.,* 2012) for the health of the mothers and their infants. The placenta's umbilical cord is the primary link between the fetus and mother. It keeps the maternal and fetal circulations separate, nourishes the fetus, eliminates fetal wastes, and produces hormones vital to pregnancy. The placenta grows throughout pregnancy and is delivered through the birth canal immediately after birth of the infant (Ashwood, 2001).

During pregnancy, the body adapts to provide a favourable environment for the developing child, modifying the nutritional needs of the mother. Pregnancy is characterized by additional energy requirements of approximately 300 kcals (or 1256KJ) per day (Kaiser and Allen, 2008) with energy metabolism changing during pregnancy and varying considerably among women (Prentice and Goldberg, 2000).

The micronutrient levels in the pregnant mothers must provide sufficient energy and nutrients to meet the mother's usual requirements, as well as the needs of the growing foetus while enabling the mother to maintain her own stores of nutrients which would be required for future breastfeeding practices. This is necessary as improved maternal levels of many micronutrients directly enhance the quality of breast milk (Picciano, 2003; Allen, 2005). Social influences such as the desire to have a lower body mass index (BMI) despite having a

BMI in the normal range (Fayet *et al.*, 2012) could negatively impact the nutrient status of women and related health outcomes. The micro and macro-nutrient deficiencies have been implicated as significant causes of various medical disorders in developing countries (Ogbodo *et al.*, 2012). Developing countries are confronted with adverse socioeconomic environment and thus, pregnant women hardly enroll for antenatal care within their first trimester, except few primigravidae (Nwagha *et al.*, 2008; Ogbodo *et al.*, 2009).

Pregnant women need additional protein for initial deposition of pregnancy related tissue and to maintain new tissue (Kathleen and Drora, 2010). When the pregnant woman's diet does not supply the required nutrients for her needs and for those of the foetus, the foetal requirements are met by withdrawing these from the tissues of the pregnant mother. The demands of the developing fetus may cause the mother to develop nutritional iron deficiency anaemia. This tissue depletion weakens the mother and increases the probability of serious complications and the chances of delivering an infant with low birth weight (LBW) who is unlikely to feed adequately early in life (Simpouolous, 1991).

A number of cross sectional, case control, and longitudinal studies reported that blood concentration of vitamin B_{12} drops during normal pregnancy. It is estimated that about 30% of pregnant mothers have some form of vitamin deficiency and 75% of women would be deficient in at least one vitamin if they were to not take supplements (Kontic-Vucinic *et al.*, 2006). A reduction in plasma vitamin B_{12} throughout the course of normal pregnancy was reported by Bruinse and van den Berg, (1995). Pardo *et al.*, (2000), Koebnick *et al.*, (2002) and Murphy *et al.*, (2007) also observed reductions in vitamin B_{12} throughout pregnancy. In a study carried out in Nigeria by VanderJagt and coworkers, they found out that 9% of the 146 pregnant women had a serum vitamin B_{12} concentration below the 148pmol/L(VanderJagt *et al.*, 2007) which is the cutoff for vitamin B_{12} deficiency (Carmel *et al.*, 2003). In a subsequent study of 98 pregnant Nigerian women, VanderJagt *et al.*, (2009) documented that 12% of the subjects had vitamin B_{12} levels in the deficiency range while 4% of the subjects had a serum folate concentration less than the cutoff for serum folate. A cross sectional study of 143 pregnant women in Jos (Nigeria) by VanderJagt *et al.*, (2011) revealed that 36% of the pregnant women were classified as vitamin B_{12} deficient. In a longitudinal study, Morkbak and coworkers observed that serum vitamin B_{12} levels decreased over the course of pregnancy from 18weeks gestation up to the time of delivery (Morkbak *et al*., 2007). A cross sectional survey in Belgium, Vandevijvere *et al*, (2012) showed that out of 1137 pregnant

women, 39% of them had folate status that might not be optimal to prevent neural tube defects.

Vitamins and minerals collectively referred to as micronutrients, are essential nutritional elements for all humans, especially pregnant women (Black, 2001). Micronutrient deficiencies in pregnant women are recognized as major public health problems in many developing countries ((IoM, 2001; WHO, 2004). Deficiencies in micronutrients such as folate, iron and vitamins are highly prevalent and may occur concurrently among pregnant women (Black *et al.*, 2008). This is because the young females are at risk of nutrient deficiencies due to poor diets and higher requirements for micronutrients, such as iron and folate especially in the periconceptional period and during pregnancy. Increased risk of low birth weight babies which affects some 20 million newborns annually, mainly in developing countries is also one of the associations of low folic acid levels during pregnancy (Metcoff, 1981, Seshadri, 2001). Micronutrients seem to affect pregnancy outcomes through alterations in maternal and foetal metabolism due to their role/involvement in enzymes, signal transduction and transcription pathways as well as in oxidative stress (McArdle and Ashworth, 1999). Interestingly, vitamin and mineral deficiencies may occur in both under-nutrition (mainly an issue in developing countries) and over-nutrition (Young *et al.*, 2004).

Okafor *et al.*, (2017) recorded decrease in mean haemoglobin, hematocrit, serum iron, serum ferritin and percent transferrin saturation while increase in total iron binding capacity (TIBC) and serum transferrin receptor (sTfR) among pregnant women in Calabar. In Port Harcourt, Amah-Tariah and coworkers showed significant reduction in serum iron and an increase in unsaturated iron binding capacity with progression of pregnancy (Amah-Tariah *et al.*, 2011). Rawal and colleagues, found an increase in sTfR levels with the progression of pregnancy among the Chinese pregnant women (Rawal *et al*., 2017). Kumar *et al.*, (2017) reported a continous rise in serum transferrin level in all three trimesters and a drop in serum ferritin level only in the second trimester. Asif *et al.*, (2007) and Bhale *et al.*, (2013) recorded a consistent decrease in serum ferritin level across the three trimesters.

Reports have suggested significant differences in the values of white blood, red blood and platelet counts in pregnant Caucasian subjects during the trimesters of pregnancy (Howarth *et al*., 1999; Ceyhan *et al*., 2006; Forhecz *et al*., 2009); reports from Nigeria have confirmed such variations in leucocyte counts (Flemming *et al.*, 1985), haematocrit, white blood cell and platelet counts (Akingbola *et al.*, 2006) during pregnancy but in a cross sectional study.

The World Health Organization indicates that, on average, 56% of pregnant women in developing countries and 18% in industrialized countries are anaemic (De Maeyer and Adiels Tegman, 1985; Allen, 2000). More than 40% of pregnant women around the world are anaemic, mostly due to iron deficiency (de Benoist *et al.*, 2008). Studies have shown that iron deficiency increases the rate of premature delivery and perinatal mortality (Rasmussen, 2001).

The prevalence of maternal under-nutrition – that is, a body mass index (BMI) of less than 18.5Kg/m^2 ranges from 10-19% in most countries (Black *et al.*, 2008). More than 20% of women in Sub- Saharan Africa, South-central and Southeastern Asia, Yemen; have a BMI of less than 18.5Kg/m^2 (Black *et al.*, 2008). In India, Bangladesh and Eritrea, 40% of women have a low BMI, which has adverse effects on pregnancy outcomes and increases the risk of infant mortality (Black *et al.*, 2008). The World Health Organization (WHO) has described the increase in obesity in many countries as a pandemic of major public health concern (WHO, 1998), and pre-pregnancy underweight a risk factor for adverse gestation outcomes (Cnattingius *et al*., 1998). Obesity also increases pregnancy complications such as gestational diabetes, hypertensive disorders, and perinatal morbidity and mortality (Wolfe, 1998).

Interestingly, the dietary habits of the urban people have been influenced by an influx of foreign workers, availability of wide range of imported goods, various fortified foods and nutrients supplements and a rise in purchasing power (Gibbon and Moyal, 1988). The prevalent traditional eating habit coupled with the novel western lifestyle is leading towards the rising incidence of obesity (Al-Shoshan, 1992). This practice has not improved the maternal and perinatal mortality indices, as they still remain embarrassingly high (Nwagha *et al.*, 2010). Several studies have been done in Owerri, Jos, Ibadan, Port Harcourt and Calabar on the levels of vitamin B_{12}, folate, iron levels and some haematologic parameters but there is a dearth of literature or data on these parameters among pregnant women across trimesters to determine their nutritional status. It has become imperative therefore to assess these parameters in a longitudinal study among the pregnant women from the urban area of Calabar, Cross River State, in the southern part of Nigeria.

1.2 Statement of the problem

Deficiencies in micronutrients such as folate, iron and vitamins are highly prevalent and may occur concurrently among pregnant women (Black *et al.*, 2008). There is an increasing evidence that the global prevalence of vitamin B_{12} deficiency, particularly in the developing

parts of the world are underappreciated (Stabler and Allen, 2004, Garcia-Casal *et al.*, 2005, Jones *et al.*, 2007, Xavier *et al.*, 2010).. The World Health Organization (WHO) indicates that, on average, 56% of pregnant women in developing countries are anaemic (Allen, 2000). Maternal under-nutrition or otherwise, a body mass index (BMI) of less than 18.5Kg/m^2constitutes 10-19% in most countries (Black *et al.*, 2008) and the increase in obesity in many countries has been described by WHO as a pandemic of major public health concern (WHO, 1998). This has made the studying of the nutritional indices of pregnant women across trimesters in urban areas deserve greater or special attention.

1.3 Aim and objectives

1.3.1 Aim

This study was aimed at assessing the nutritional indices in the pregnant women across the trimesters using some haematologic parameters.

1.3.2 Objectives

In order to achieve the aim, the following objectives were formulated;

1. To determine the levels of total protein, albumin, vitamin B_{12}, and folate across trimesters in pregnant women attending antenatal care at the University of Calabar Teaching Hospital, (UCTH), Calabar and non pregnant women (control).
2. To determine the iron levels (serum iron, unsaturated iron binding capacity, total iron binding capacity, ferritin, serum transferrin receptors and transferrin saturation) across trimesters in the pregnant women.
3. To assess haematologic parameters (haemoglobin, packed cell volume, red cell indices, total white blood cell count, platelets, absolute and percentage white blood cell count) across trimesters in the pregnant women.
4. Determine the influence of age, educational status, parity and income of the pregnant women on the indices measured.
5. To correlate ferritin concentration with the other iron status parameters and with total protein levels, albumin, folate, vitamin B_{12}, and BMI.

1.3 Justification for the study

Vitamins and minerals collectively referred to as micronutrients, are essential nutritional elements for all humans, especially pregnant women (Black, 2001). Micronutrient deficiencies in pregnant women are recognized as major public health problems in many developing countries ((Institute of Medicine 2001; WHO, 2004). Nutritional deficiencies contribute significantly to the high rates of mortality and morbidity among pregnant women

in developing countries. Interestingly, vitamin and mineral deficiencies may occur in both under-nutrition (mainly an issue in developing countries) and over-nutrition (Young *et al*., 2004). In Nigeria, there's a high prevalence of both under and over nutrition, as well as nutrient deficiencies including iron, folate and vitamins (Lindsay *et al.*, 2012). Lack of adequate nutrition of pregnant women as well as the growing foetus is a key causal factor for stillbirths prior to the onset of labour (Lawn *et al.*, 2009); it impairs foetal brain development and cause abnormalities in endocrine functioning, organ development and the energy metabolism of the child (Shieh and Carter, 2011). Studies have shown that iron deficiency increases the rate of premature delivery and perinatal mortality (Rasmussen, 2001). More than 40% of pregnant women around the world are anaemic, mostly due to iron deficiency (de Benoist *et al.*, 2008). Studying the nutritional indices of pregnant women across the trimesters therefore, will provide information on the management of pregnant women in Calabar thereby ensuring healthy pregnancy outcome.

1.5 Significance of the study

With increasing awareness of the importance of adequate nutritional status in normal pregnancy, pregnant women were assessed for vitamin B_{12}, folate, total protein, albumin, iron levels and haematologic parameters. The comparison of the values within the trimesters among the pregnant women and also with the non pregnant women, including the relationship between the parameters was elucidated. The outcome of the findings in particular will help in the management of pregnant women and in the health care delivery policy making in general. It therefore, forms a baseline data for further research.

1.6 Research questions

1. Is there any difference in the levels of total protein, albumin, vitamin B_{12}, and folate across trimesters in normal pregnant and non pregnant women?
2. Is there any difference in the iron levels (serum iron, unsaturated iron binding capacity, total iron binding capacity, ferritin, serum transferrin receptors and transferrin saturation) across trimesters in normal pregnant and non pregnant women?
3. Is there any difference in the levels of the haematologic parameters (haemoglobin, packed cell volume, red cell indices, total white cell count, platelets, absolute and percentage white cell count) across trimesters in normal pregnant and non pregnant women?
4. Is there any association between the demographic characteristics and the indices measured?

5. Is there any correlation in the maternal ferritin concentration of the pregnant women and the indices measured?

1.7 Research Hypothesis (Null)

1. There is no difference in the levels of the total protein, albumin, vitamin B_{12}, and folate across trimesters in normal pregnant and non pregnant women.
2. There is no difference in the iron levels (serum iron, unsaturated iron binding capacity, total iron binding capacity, ferritin, serum transferrin receptors and transferrin saturation) across trimesters in normal pregnant and non pregnant women.
3. There is no difference in the levels of the haematologic parameters (haemoglobin, packed cell volume, red cell indices, total white cell count, platelets, absolute and percentage white cell count) across trimesters in normal pregnant and non pregnant women.
4. There is no association between the demographic characteristics and the indices measured.
5. There is no correlation between the maternal ferritin concentration of the pregnant women and the indices measured.

1.8 Scope of the Study

The present study was carried out on apparently healthy women with normal pregnancy. The pregnant women were those in their first trimester attending antenatal care at the UCTH, Calabar, not taking therapeuitic diets and who are free from diabetes mellitus, sickle cell anaemia and human immunodeficiency virus. Those who met the set criteria were involed in the study and were assessed for the nutritional indices throughout the course of the pregnancy. Thus, results of this study may be used in the assessment of the health status of normal pregnant women at different trimesters.

CHAPTER TWO

LITERATURE REVIEW

2.1 Nutrition and pregnancy

Nutrition is the science that interprets the interaction of nutrients and other substances in food in relation to maintenance, growth, reproduction, health and disease of an organism. It includes food intake, absorption, assimilation, biosynthesis, catabolism and excretion. It is the supply of materials that organisms and cells require to live. By practicing a healthy diet, many of the known health issues can be avoided (Sahoo and Panda, 2006).

Malnutrition refers to insufficient, excessive, or imbalanced consumption of nutrients by an organism. In developed countries, the diseases of malnutrition are most often associated with nutritional imbalances or excessive consumption. In developing countries, malnutrition is more likely to be caused by poor access to a range of nutritious foods or inadequate knowledge. Malnutrition resulting from inadequate dietary intake is associated with growth failure and development of protein-energy malnutrition, especially during the gestation (Kathleen and Drora, 2010).

The globalized high-energy and low-nutrient density Western dietary patterns and trends, typified by snacking, breakfast skipping, fast foods, soft drinks and convenience foods, are nutritionally unbalanced, and intake of micronutrients in general fails to meet recommended daily allowance (RDA) values (Paeratakul *et al.*, 2003; Drewnowski and Spencer 2004; Cordain *et al.*, 2005). Recognition of nutrient deficiencies in women of reproductive age is important not only because nutritional status affects women's health and wellbeing, but also because deficiencies are associated with adverse pregnancy outcomes. Also, deficiencies in micronutrients such as folate, vitamin B_{12}, iron and trace elements can have adverse consequences on infant mortality and morbidity. Maternal micronutrient deficiencies resulting from the expense of, and/or lack of access to foods rich in multiple micronutrients in low income earners is related to birthweight (Kramer *et al*., 2000).

Nutrition may play a role in altering the development of the placenta. In fact, despite an initially normal growth trajectory, fetuses may have impaired growth in the second part of gestation subsequent to nutrient deprivation occurring early in gestation. Early in pregnancy the muscular maternal spiral arteries are transformed to fibrinoid lined vessels, and after further invasion by endovascular cytotrophoblasts, these vessels bath the chorionic villi in

maternal blood bearing oxygen and nutrients for fetal development. Defects in this process have been reported in pregnancies complicated by intrauterine growth restriction (IUGR) (Sibley *et al.*, 2005; Cetin and Alvino, 2009).

The process of implantation and placentation may also be affected by maternal nutrition. Placental function is critical for nourishing the fetus throughout pregnancy (Cross and Mickelson, 2006; Jansson and Powell, 2006). Specifically, the placenta forms a highly branched villous structure thus providing nutrients and oxygen to the fetus (Cetin *et al.*, 2005) to assure appropriate fetal growth (Sparks *et al.*, 1998). In particular, intrauterine growth restriction (IUGR) is associated with a range of alterations in placental transport functions, whereas accelerated fetal growth, in association with maternal diabetes, is characterized by increased activity of placental systems (Sacks, 2004; Cetin and Alvino, 2009).
For this reason, micronutrient malnutrition represents an important topic of public health worldwide, mainly in vulnerable population group such as pregnant women. There is sound evidence that adequate intake of micronutrients can prevent many serious birth defects, reduce the risk of premature and low birthweight (LBW) infants, and support maternal health.

In particular, the pre-conception period is critical in determining fetal development and health. The onset of several malformations and pregnancy related disorders (i.e. congenital abnormalities, fetal loss, miscarriage, insufficient fetal growth, premature birth and pre-eclampsia) may indeed occur during this period (Steegers, 2005). Since pregnancy is characterized by different stages that represent a continuum, the timing of a nutritional insult impacts differently both on the overall outcome of pregnancy and on the nature of adult diseases by programming the post-natal pathophysiology, and having the potential to affect cell numbers or differentiation in the developing embryo (Newnham *et al.*, 2002; Rhind, 2004; Buckley *et al.*, 2005; De Boo and Harding 2006). Each stage in embryonic and fetal development is indeed strongly influenced by maternal nutrients and hormones (Ashworth and Antipatis, 2001; Gluckman *et al.*, 2008), and the placental–maternal—fetal somatotropic axes are fundamental in modulating this interaction (Gluckman and Pinal, 2002). Therefore, the adequate intake of vitamins and minerals such as iron, vitamin B_{12} and folate is essential for optimal health.

2.2 Anthropometric measurements in pregnancy

Anthropometric measurements, among the most frequently applied methods for assessing nutritional status in pregnant women, are recognized as effective tools in the prevention of

perinatal morbi-mortality, the prognosis of child health, and the promotion of women's health. In addition, their easy application, low cost, and non-invasive nature reinforce their viability as a nutritional assessment method. The World Health Organization (2002; 2006) cites maternal anthropometric aspects and intake of adequate nutrients as determinants of fetal growth, demonstrating a close association with these parameters and weight and gestational age at birth. The finding of the nutrition and food security survey showed that 13.4% of pregnant women are underweight. Further study found that the study sample's mean weight and BMI at the first visit to the clinic were 52.89 kg and 22.1kgm-^{2}. Several studies have shown an association between anthropometric indicators and pregnancy outcome. Both insufficient and excessive gestational weight gain are strongly associated with maternal- fetal complications such as gestational diabetes, hypertensive pregnancy disorders (HPD), macrosomia, and low birth weight (Olson *et al.*, 2004; Stotland *et al.*, 2005; Mohanty *et al.*, 2006). It is well known that nutritional intervention focus on women's health during the reproductive stage-not only in the preconception period but also during the prenatal period, and culminating in assistance to lactating woman, helps achieve adequate newborn nutritional status and is reflected in childhood health and nutritional conditions (WHO, 2006). In terms of anthropometric assessment, pregnancy is characterized as a brief observation period in which the anthropometric index undergoes rapid changes (WHO, 1995). Conducting anthropometric measurements during the prenatal period is a routine practice. However, its actual effectiveness depends on the availability of services, the number of prenatal care visits, and women's consent to having their measurements taken. Ensuring the most beneficial outcome requires the use of anthropometric assessment methods that help develop an effective and practical application instrument capable of predicting maternal and infant health conditions and allowing for adequate nutritional intervention during this period of high biological vulnerability (WHO, 1995). Nonetheless, the use of anthropometric measurements in pregnancy to promote health and improve obstetric results-including maternal morbi-mortality indices, conditions at birth, and perinatal mortality, raises certain issues that underscore the need for validation of some of the practical aspects of the process as well as its universal applicability in normative terms (Brennand *et al.*, 2005; Kruger, 2005; Cedergren, 2006; Ochsenbein-kolble *et al.*, 2007). These issues include: water retention, which frequently causes edema; alteration in body composition; and postural, hormonal, and other physiological conditions of pregnancy that are directly reflected in weight gain, the most common anthropometric measure used during pregnancy follow-up. Measurements of weight and height, and their associations, to produce pre-gestational body mass index (BMI)

indices, and (less commonly) the calculation of arm circumference and cutaneous folds, are also recommended in the anthropometric nutritional assessment of pregnant women (WHO, 1995).

2.3 Macronutrients and pregnancy

In Western settings, studies have shown links between the balance of macronutrients in women's diets and the size of newborns. Timing appears to play a critical role in this- scientists believe there are critical windows in pregnancy during which maternal diets are particularly important (Moore and Davies, 2005). One example of this is the balance of energy and protein in women's diets. Evidence from past studies has shown that balanced energy and protein supplement seems to help improve foetal growth and reduce the risk of foetal/neonatal death. However, high protein diets alone and energy/protein restriction in overweight and obese mothers have been found to have no benefits on pregnancy outcome and could actually be harmful to infant's health.The macronutrient needs of pregnant women are not considerably different to that of non-pregnant women. On the basis of the best evidence most women only need to increase their energy intakes in the third trimester of pregnancy by about 180 Kcals/day. High quality proteins should be consumed throughout pregnancy (about 50g/day) and include lean meat, milk, certain cheeses and cooked eggs and intakes of trans fats should be kept to a minimum. To achieve recommended levels of intake of polyunsaturated fatty acid (PUFA), women should be advised to eat 2 portions of fish per week (one oily) but avoid certain fish species such as shark, marlin and swordfish.

2.3.1 Carbohydrate and pregnancy

2.3.1.1 Carbohydrate metabolis

In healthy mothers, the secretion of insulin may be 50-70% lower in pregnancy compared to levels in non-pregnant mothers (Butte, 2000). Although reduced levels of insulin also known as 'insulin resistance' appear to serve a biological purpose -to shunt (divert) ingested nutrients particularly glucose) to the foetus. Hypoglycaemia may also contribute to the development of gestational diabetes mellitus in late pregnancy. When combined with the rising rates of obesity, gestational diabetes mellitus is becoming an increasingly frequent occurrence in pregnancy (Sathyapalan *et al.*, 2010).

2.3.1.2 Insulin sensitivity

Levels of insulin insensitivity are a tale of two stories in pregnancy. In the early stages of pregnancy, muscle, fat and liver cells are more responsive to the hormone insulin. As a result,

plasma glucose, amino acids, and free fatty acid levels reduce and glucose is synthesized. However, 15-30% increase (responsiveness) can be made in late pregnancy. Glucose is transferred from the mother's blood stream, across the placenta and to the foetus with the aid of glucose transporters known as GLUT 1 AND 4. These transporters play an important role in ensuring that the foetus receives a constant supply of energy.

When a mother is hypoglycaemic, these transporters are up-regulated and when the mother is hyperglycaemic, this can be down-regulated (Hay, 2006a). These homeostatic mechanisms help to ensure that the foetus is provided with a steady supply of glucose. Once the glucose passes across the placenta, this is then converted to liver/muscle glycogen or storage lipids, which will help to maintain glucose homoestasis in the infant after delivery. Although the foetus has the ability to adapt to changes in glucose supply to some extent, such changes may underlie (be the cause or basis of) certain metabolic disorders such as insulin resistance, obesity and diabetes mellitus later in life (Hay, 2006b).

2.3.2 Lipid metabolism

Pregnancy has a profound effect on lipid metabolism. Most recently, the use of stable, non-radioactive isotope together with glucose and insulin clamps has enabled the field of lipid metabolism to be studied in detail (Butte, 2000). Overall, studies have shown that fat accrues during the first two thirds of pregnancy but then switches in the last trimester when fat reserves are mobilized to provide a supply of free fatty acids to the foetus. Therefore, blood lipids levels are usually elevated in the later stages of pregnancy (Herrera, 2002b).

During pregnancy, most of the subcutaneous fat is deposited centrally (between the mid-thorax and mid-thigh). This was demonstrated in a large follow-through study undertaken by Sidebottom *et al.*, 2001. Lippi *et al.*, 2007 undertook a comprehensive lipid and lipoprotein analysis from 57 women each at the different phases of pregnancy. Lipid profile varied considerably when women from different trimesters of pregnancy were compared. Total cholesterol, low density lipoprotein and high density lipoprotein cholesterol levels were all significantly higher later in pregnancy when compared to samples taken in early pregnancy or from non-pregnant controls.

Similar findings have been reported amongst women from developing regions. In Bangledesh, total cholesterol, high density lipoprotein and low density lipoprotein levels

were also elevated in the second and third trimesters of pregnancy when compared to serum levels analysed from non-pregnant women (Hussain *et al.*, 2009).

A wealth of evidence from epidemiological and experimental studies have shown that fibre-rich diets play an important role in preventing chronic diseases such as diabetes, coronary heart disease, obesity and disorders of the bowel, although conflicting definitions can make interpretations from such studies difficult (Mann and Cummings, 2009).

2.4 Protein and pregnancy

Proteins are structural materials in much of the animal body (e.g. muscles, skin, and hair). They also form the enzymes that control chemical reactions throughout the body. Each protein molecule is composed of amino acids, which are characterized by inclusion of nitrogen and sometimes sulphur. As there is no protein or amino acid storage provision, amino acids must be present in the diet. The body can synthesize some amino acids, whereas the indispensable formerly known as the essential amino acids must be obtained from food. This includes foods such as lean meat, chicken, oily fish, and dairy products.

Protein is also found in whole grains and vegetables and whilst these are not high in proteins they can help contribute to total daily protein intakes. Protein forms an essential component of a healthy diet in humans to support both growth and maintenance. Protein in the body plays structural (keratin, collagen) and functional (enzymes, transport proteins, hormones) roles (Institute of Medicine, 2005; WHO, 2007). Ultimately, most mammalian protein is composed of 20 different amino acids, and thus there is a need in our body for both an adequate supply of amino acids and total protein (nitrogen).

Pregnant women need additional protein for initial deposition of pregnancy related tissue and to maintain new tissue in order to prevent growth failure and development of protein-energy malnutrition during the gestation (Kathleen and Drora, 2010). It is needed for physical growth and cellular development of the baby as well as the expansion of the placenta and maternal tissues. Extra protein is also needed to support the formation of red blood cells and circulating proteins especially as women's blood volume increases in pregnancy.

Ideally, diets should contain adequate but not excessive levels of protein. Although a certain level of intake is needed in pregnancy, high intakes in pregnancy may lead to unfavourable pregnancy outcomes. A study has shown that women who consumed high protein diets in pregnancy and delivered large infants had children with a lower ponderal index (Andreasyan

et al., 2007). As the ponderal index is a measure of lean body mass, the results from this study imply that high protein diets may lead to changes in infant body composition.

Scientists have also followed up women who ate high meat, low carbohydrate diets over 30 years ago. Researchers found that women who ate more meat and fish in the second half of pregnancy had offspring with a higher systolic blood pressure in adulthood (Shiell *et al.*, 2001). Authors concluded that high protein diets may induce a state of metabolic stress that could affect long-term health of the offspring. Throughout the course of pregnancy, it has been advised that women eat around 51g of protein each day. Vegetarian and vegan mothers, in particular should make sure they are consuming enough foods that provide protein throughout their pregnancies.

2.4.1 Protein requirement in pregnancy

The current definition of protein requirement is as follows: "the lowest level of dietary protein intake that will balance the losses of nitrogen from the body, and thus maintain the body protein mass, in persons at energy balance with modest levels of physical activity, plus, in children or in pregnant or lactating women, the needs associated with the deposition of tissues or secretion of milk at rates consistent with good health" (WHO, 2007). Thus, during pregnancy, an exceptional stage of life defined by rapid growth and development and enormous maternal physiologic changes from the time of conception to birth, adequate dietary protein is crucial to ensure a healthy outcome. Within several weeks of conception, adjustments in protein metabolism occur to support fetal growth and development while maintaining maternal homeostasis and preparing for lactation (King, 2000). Protein utilization from foods and deposition as new tissues are energy dependent at stages of absorption, amino acid transport, protein synthesis, and proteolysis. Thus, dietary intake during pregnancy must have sufficient energy and protein to ensure the full-term delivery of a healthy infant. The additional energy required during the full term of pregnancy has been estimated to be approximately 77,000 kcal (WHO, 2004), although the energy cost of pregnancy is not distributed equally throughout the gestational period. This is because the amount of protein deposited in maternal and fetal tissues varies during pregnancy, with non significant deposition during the first trimester, gradually increasing during the second trimester, and with most occurring in the third trimester (Butte and King, 2005).

During pregnancy, protein availability is a key determinant of fetal growth. Protein requirements increase in pregnancy to support maternal tissue synthesis and foetal growth, principally in the third trimester. When foetal growth is restricted, this may have implications

of cardiovascular health and renal function in the longer term, particularly at the population level (Geelhoed and Jaddoe, 2010). Protein needs are increased as early as 16 wk of gestation, (Elango and Ball, 2016) although it was previously thought that the demand for protein would be low initially and increase substantially only by late pregnancy (King, 2000). There seems to be a metabolic adaptation in pregnancy that enhances the efficiency of protein synthesis from the start of pregnancy (Duggleby and Jackson, 2002).

Low protein diets are associated with adverse outcomes of pregnancy. Protein intakes of less than 75g per day have been associated with low birthweight and birth length (IoM, 2002), and intakes of less than 50g per day is also associated with increased maternal morbidity. However, there does not seem to be a straightforward relationship between maternal total protein and birthweight (Duggleby and Jackson, 2002). High protein intakes (over 20 percent of total energy) may have adverse effects on birthweight and should be avoided (Rush *et al.*, 1980). Ammonia and urea are produced in protein metabolism, and the foetus has limited ability to detoxify ammonia and excrete urea, particularly in the first trimester. For the foetus to grow efficiently, amino acids need to be transported across the placenta effectively.

The relationship between breast milk protein content and maternal diet and nutritional status is inconclusive. Some studies have found lower levels of protein in the breast milk and colostrum of malnourished women, while others found similar levels (Emmett and Rogers, 1997). Protein supplementation in the mother has resulted in small increases in milk protein in some studies (seen in well-nourished women when increasing protein from 8 to 20percent of energy), while others found daily protein milk output did not change, but milk volume increased and protein concentration dropped (Goppala and Puri, 1992). Even though maternal dietary intake of protein has little effect on breastfeeding performance, protein intake of over 1g per kg day conserves maternal lean body mass (Motil *et al.*, 1996). In the first trimester of pregnancy, there is no additional protein requirement, so the recommended dietary intake (RDI) for protein for pregnant women aged 19-50 years in the first trimester is 40g per day (0.75g/kg/day). In the second and third trimesters, the RDI for protein for pregnant women aged 19-50 years is 60 g per day (1.00g/kg/day), and for women aged 14-18 years the RDI is 58g per day (1.02g/kg/day).

Immune proteins are particularly significant in their role in preventing immune-related complications including infections and infestations though the development of immunity, especially against malaria, is said to increase with parity (Riley *et al.*, 1989; Rasheed *et al.*, 1993; Rogerson *et al.*, 2007). Albumin is the fraction that is usually lost during proteinuria in

pregnancy because of its molecular weight. Thus, hypoalbuminemia in pregnancy may not necessarily be only due to decrease in production but also dilution (due to increased volume) and increased loss in urine (proteinuria). These alterations to the immune status of pregnant women though this is required to enable mothers tolerate genetically different fetal tissues during pregnancy, but may also increase susceptibility of the pregnant women to infections (Yip *et al.*, 2006).

Some studies show that rates of protein turnover may be related to birth outcome. One study found that women with a higher lean body mass and higher rates of protein turnover 18 weeks into pregnancy had babies that were longer at birth (26% variation in length) (Duggleby and Jackson, 2001). The same authors also identified that heavier infants are born to mothers who had lower levels of amino acid turnover in pregnancy, even after adjustments were made for length of pregnancy and the infants gender ((Duggleby and Jackson, 2002). Overall, it appears that rates of amino acid oxidation vary widely between pregnant mothers and may influence pregnancy outcomes, including birth weight. Duggleby and Jackson (2001, 2002), have shown a relationship between increase in synthesis and weight and length at birth. It is in the third trimester that the growth in foetal protein mass is most rapid and Naismuth *et al.*, (1982) from experiments in rats, made the interesting suggestion that in the second trimester, the mother stores protein in muscle which is drawn upon in the third trimester to support the growth of the foetus (Naismuth *et al.*, 1982). The rate of intravascular albumin synthesis is also substantially increased in late pregnancy (Olufemi *et al.*, 1991). According to Whittaker *et al.*, (2000), pregnancy did not alter the sensitivity of protein breakdown to insulin. The second adaptation is a decrease in amino acid oxidation and urea synthesis, particularly in the first and second trimesters. Changes in protein and nitrogen metabolism occur in early pregnancy, presumably in response to pregnancy related hormones (Kalhan, 2000). Protein turnover on a weight basis, however, does not change (Kalhan, 2000). Serum total protein and albumin fall progressively and by term are 30% lower than in non -pregnant values (Hytten, 1991).

The process of amino acid transfer to the foetus across the placenta is complex. Overall, levels of amino acids in maternal plasma are lower than in foetal plasma, causing amino acids to move against a concentration gradient. The movement of amino acid across the placenta is facilitated by a combination of transporters and exchangers. Together, they ensure that foetal cells are supplied with amino acids that will ultimately support foetal growth (Cleal and Lewis, 2008).

2.5 Water and pregnancy

Pregnant women have different hydration needs when compared to others in the population. Water requirements are around 300ml higher in pregnancy. Women should drink fluids on a regular basis, even before they feel thirsty as thirst sensations usually occur when body water levels are already reduced. Ideally, water should be the main source of fluids, followed by warm beverages milk, low calorie soft drinks, fruits juices. On a final note, moisture-rich foods can also contribute to daily water intakes. It is normally assumed that the contribution of food to total water intake is 20-30% whereas 70-80% is provided from beverage sources.

A study from the Danish National Birth Cohort has found that drinking artificially sweetened carbonated and non-carbonated soft drinks in pregnancy can increase preterm delivery risk. Preterm deliveries (delivering a baby before 37 weeks into pregnancy), are one of the most common medical complications in pregnancy and leading cause of infant morbidity and mortality (Khashu *et al.*, 2009).

Tea consumption and low intake of red meat were associated with anemia (Wolmarans *et al.*, 2003; Baig-Ansari *et al.*, 2008; Pasricha *et al.*, 2008). Although this has not been studied before on a large scale, high consumption of products containing saccharine have been found to aggregate on the foetal side of the placenta (London, 1988).

Compared to women not drinking artificially sweetened carbonated soft drinks, those drinking 4 servings or more of artificially sweetened carbonated soft drinks on a daily basis had a significantly higher risk of delivering prematurely (Halldorsson *et al.*, 2010).

2.6 Vitamin and pregnancy

Vitamins are essential to maintain normal metabolic processes within the body. Certain vitamins deficiency may be noticed in several days (except vitamin B12) and vitamin C deficiency symptoms may become apparent within weeks. Other vitamins are stored in greater amounts, particularly the fat soluble vitamins. Scientists have calculated that vitamin D reserves may last for up to 2months and vitamin A stores up to 5 months (Bsoul and Terezhalmy, 2004). Clearly, every individual has different levels of vitamin stores and rates of depletion will vary depending on dietary quality and whether supplements are taken.

Folate and vitamin B_{12} deficiencies occur primarily as a result of insufficient dietary intake or, especially in the case of vitamin B_{12} deficiency in the elderly, poor absorption. High folate intake can mask anaemia caused by B_{12} deficiency, delaying diagnosis of B_{12} deficiency and increasing the risk of permanent neurological damage to the foetus (Shane and Stokstad, 1983). However, at the recommended folic acid tablet dose, the masking effect does not

appear to be a significant issue, particularly as B_{12} deficiency anaemia tends to affect predominantly older people and is usually not a problem in women of child bearing age (unless they are vegans). B_{12} vitamin is mostly found in foods of animal origin and deficiency was not reported as a frequent cause of anemia.

Deficiency is certainly more prevalent in strict vegetarians, but lacto-ovo vegetarians are also at higher risk for inadequate intakes. If the mother is folate-depleted during lactation, breast-milk concentrations of the vitamin are maintained while the mother becomes more depleted. In contrast, vitamin B_{12} concentrations in breast-milk can be markedly lower in vitamin B_{12}-depleted women (WHO, 2008).

There are many reasons why vitamin deficiencies may occur in pregnancy. Insufficient nutrient stores, age upon conception, availability of food, level of education, individual food choices and season may all influence a woman's nutritional status. For example research in Nepal has shown that the diet quality of pregnant women is generally better in Winter months before the hot Summer and monsoon seasons reduce food supplies and vitamin deficiencies which are often multiple become common (Jiang *et al.*, 2005).

Physical and strenuous work and work related stress could contribute to maternal stressors and these are capable of giving rise to poor pregnancy outcome. It is important to consider that women's diet quality and health in their reproductive years can affect health in pregnancy and in turn, the health of the next generation leading to a cycle of poor health.

2.6.1 Vitamin B_{12} or cobalamin and pregnancy

Cyancobalamin is the only B vitamin to contain a mineral (cobalt) and is one of the largest and most complex of the B vitamins. Vitamin B_{12} exists in a number of different chemical forms. The molecule consists of two halves: a "planar group" and "nucleotide" set at right angles to it (Hoffbrand and Green, 2005). The planar group is a corrin ring and the nucleotide consists of the base, 5, 6-dimethy enziminazloe, and a phosphorylated sugar, ribose-s-phosphate (Hoffbrand and Green, 2005). In nature, the vitamin is mainly in the 5'-deoxyadenosyl (ado) form. This is the main form in human tissues and is located in the mitochondria. It serves as the cofactor for the enzyme methylmalonyl CoA mutase. The other major natural cobalamin is methylcobalamin, the main form in human plasma, as well as the cytosolic form in cells. It serves as the cofactor for the enzyme methionine syntase. Cobalamin is present in animal protein and absorbed in the terminal ileum. R-protein (haptocorrin), secreted by salivary glands, binds cobalamin in the stomach and transports cobalamin to the duodenum where pancreatic proteases degrade the R-protein. Cobalamin is

then released and binds to intrinsic factor released from gastric parietal cells. The cobalamin intrinsic factor complex subsequently binds to receptors on ileal enterocytes. Vitamin B_{12} is synthesized by solely micro-organisms, and therefore predisposes vegetarians, a common dietary practice among young women, to a greater risk of deficiency (O"Leary and Samman, 2010).

Bacteria and algae synthesize vitamin B_{12}, and it enters the human food chain through incorporation into food of animal origin such as liver, milk, meat, oocytes (WHO, 2004b). The highest amounts are found in liver and kidney (up to 100microgramme/100gramme) but it is also present in shellfish, organ and muscle meats, fish, chicken and dairy products- eggs, cheese and milk which contain small amounts (6microgramme/L) (Hoffbrand and Green, 2005). Cooking does not usually destroy cobalamin. A normal Western diet contains between 5 and 30 microgramme cobalamin daily. Adult daily losses mainly in the urine and faeces are between 1and 3 microgramme and the body does not have the ability to degrade cobalamin, daily requirements are also about 1 and 3microgramme. Vitamin B_{12} and folate are involved in single-carbon transfer and DNA synthesis, and are particularly important for young women. Long-term, vitamin B_{12} deficiency impairs cognitive function and it is important in the prevention of neural tube defects (NTD) (O"Leary and Samman, 2010).

Vitamin B_{12} is a cofactor in the metabolic transformation of homocysteine to methionine, a reaction that also requires folate (O"Leary and Samman, 2010). Low vitamin B_{12} levels are related to hyperhomocysteinaemia (HHCY) and high methylmalonic acid. HHCY has been implicated in adverse pregnancy outcomes such as placental abruption or infarction and pre-eclampsia (Tamura and Picciano, 2006; Braekke *et al.*, 2007).

Serum vitamin B_{12} is a biomarker of vitamin B_{12} deficiency, and the metabolites, methylmalonic acid (MMA) and homocysteine are functional indicators (O'Leary and Samman, 2010). Looking to surveys, a cut-off of >210nmol/L has been used in the large American National Health and Nutrition Examination Survey (NHANES) as an indicator of vitamin B_{12} deficiency (Pfeiffer *et al.*, 2005). Recently, researchers have shown increased interest in the role of vitamin B_{12} as a measure to prevent NTDs alongside folic acid. There is now good evidence that vitamin B_{12} deficiency may be associated with the development of NTD and possible cause of preterm deliveries (Molly *et al.*, 2008). Reduced vitamin B_{12} intake in infancy (as a result of inadequate maternal intake and stores) may also contribute to

range of neurological symptoms including irritability, failure to thrive, apathy, loss of appetite and delayed development (Dror and Allen, 2008). At present, UK dietary Reference Values for vitamin B_{12} are only targeted at women in general (1.5ug/day) and set at 2.0ug/day for lactating mothers. The European Union (EU), recommends 1.6 and 1.9 ug/day vitamin B_{12} for pregnant and lactating mothers respectively. Daily recommendations for non-European countries are slightly higher (2.2-2.8 ug/day) (IoM, 1998). Because of the lack of clearly defined adverse effects, SULs for vitamin B_{12} have not yet been established. The bioavailability of vitamin B_{12} from meat may range from 42% - 66% but may be less than 9% in eggs sources (Watanabe, 2007). Consequently, common causes of vitamin B_{12} deficiency include a vegetarian/ vegan diet, low intake of animal foods or malabsorption (possibly as a result of Helicobacter pylori infection) (Allen, 2009). As plant foods only contain traces of vitamin B_{12} for these risk groups (Watanabe, 2007). The dietary source of vitamin B_{12} strongly influences its bioavailability. In a study of the sources of vitamin B_{12} and their association with plasma vitamin B_{12} levels, Vogiatzoglou and coworkers determined that the bioavailability of vitamin B_{12} was greater from dairy products and fish than from meat (Vogiatzoglou *et al.*, 2009). In the dietary study they conducted in Jos, Nigeria (Bor *et al.*, 2010), only 10% of the protein in the diets of the women was derived from eggs and milk, with 25% of protein coming from meat.

Vitamin B_{12} is essential for normal blood and neurological function. Absorption of vitamin B_{12} may increase in pregnancy, and the foetus is dependent on maternal dietary intake. The placenta preferentially transports newly absorbed vitamin B_{12} from the mother's dietary intake rather than stored vitamin B_{12} from the mother's liver. The placenta then concentrates vitamin B_{12} and transfers it to the foetus. Hence, if dietary intake is inadequate, transfer to the foetus may be compromised even though the mother shows no overt signs of deficiency. Earlier studies by Luhby and colleagues showed that newly absorbed maternal B_{12} is more readily transported to placenta than maternal liver stores (Luhby *et al.*, 1958). Women who are vegan or with a low intake of vitamin B_{12} may compromise the foetal vitamin B_{12} levels even if they have become vegan only recently (Specker *et al.*, 1990).

Intriguingly, the Pune Maternal Nutrition Study (PMNS), carried out in India is one of the first studies to investigate the relationship between maternal nutrition and the offsprings risk of developing type 2 diabetes and cardiovascular disease. Results showed that over 2/3 of mothers had low B_{12} status in pregnancy (defined as circulating levels <150pmol/L) and 30% had raised homocysteine levels. Infants at 6 years old, born to mothers with low maternal vitamin B_{12} status were more likely to be insulin resistant. Interestingly, offspring to mothers

with a high folate but low B_{12} status were most likely to be insulin resistant meaning that they had a higher risk of developing diabetes of cardiovascular later in life (Yajnik *et al.*, 2008). Overall, the findings from this study are important because they demonstrate that the offspring needs an adequate supply of folate and vitamin B12 for good health. The authors speculated that vitamin B_{12} deficiency prevents the generation of methionine from homocysteine by trapping folate as 5-methyltetrahydrofolate, and subsequently reduces protein synthesis and lean tissue deposition (Yajnik *et al.*, 2008). Moreover, the increased lipogenesis might be caused by the inhibition of β-oxidation, owing to elevated concentrations of methylmalonic-co-enzyme A. In fact, it can be hypothesized that imbalances of adequate folate methyl donor and poor vitamin B_{12} cofactor lead to further depletion of mitochondrial cobalamin stores, thereby leading to dysfunction of other B_{12}-dependent reactions (Rosenberg, 2008). On the whole, these results postulate that the defects in the one-carbon metabolism play a crucial role in intrauterine programming of adult diseases.

2.6.1.1 Vitamin B_{12} requirements during pregnancy

The demand for vitamin B_{12} increases during pregnancy due to rapid cell multiplication resulting from the uterine enlargement, placental development and foetal growth. Animal and human studies suggest that absorption of the vitamin may become more efficient during pregnancy (Brown *et al.*, 1977). However, despite the increased efficiency of absorption, total plasma B_{12} declines steadily throughout pregnancy commencing with the first trimester (Fernandes-Costa and Metz, 1982). This gradual physiologically normal decline in the plasma B_{12} is thought to be due to several factors such as haemodilution, hormone fluctuations, impaired renal function, altered concentration of binding proteins (transcobalamin and haptocorrin) or active transport of vitamin B_{12} across the placenta (Guerra-Shinohara *et al.*, 2004; Obeid *et al.*, 2006). The lowest concentration is observed during the third trimester and it returns to prepregnancy levels within a few weeks postpartum. Vitamin B_{12} is actively transported to the foetus which has a significant influence for the progressive decline of maternal vitamin B_{12} levels during pregnancy. Foetal demand for the vitamin has been estimated to approximately 0.3ug/day. The well nourished human adult has about 2-5mg of the vitamin, the majority being stored in the liver which is adequate without repletion for 3-5 years. A healthy pre-pregnancy body store of B_{12} is, therefore, sufficient to meet increased demand during pregnancy (Dror and Allen, 2012).

Measurement of the total vitamin B_{12} concentration in plasma is the usual method for assessing vitamin B_{12} status. However, neurological and hematological symptoms of deficiency can occur in individuals with plasma vitamin B_{12} concentrations in the low-normal range (Karnaze and Carmel, 1990). Conversely, some individuals with low vitamin B_{12} concentrations remain symptom-free. Moreover, the plasma vitamin B_{12} concentration is not a reliable indicator of vitamin B_{12} status in pregnancy since there is a gradual, physiologically normal decline in the plasma concentration of vitamin B_{12} during an uncomplicated pregnancy (Koebnick *et al.*, 2002; Milman *et al.*, 2006).

2.6.1.2 Vitamin B_{12} deficiency

The first sign of B_{12} deficiency is characterized by a decrease in serum holoTC, after which both methylmalonic acid (MMA) and plasma total homocysteine (tHcy) start to increase, and finally there is a reduction in serum vitamin B_{12}. The next stages of negative B_{12} balance is impaired erythropoiesis, accompanied by yet lower concentrations of holoTC and serum B_{12} and hypersegmented neutrophils. In the end, haemoglobin concentrations are reduced which results in macrocytic anaemia (Herbert, 1994). Emerging data suggest that deficiency of vitamin B_{12} indicated as a serum or plasma concentration of vitamin B_{12} lower than 200pg/ml or when serum levels of methylmalonic acid are elevated (Allen, 2009) are highly prevalent in women of reproductive age, particularly amongst populations with limited intake of animal source foods. It is difficult to quantify the prevalence deficiency in pregnant women partly due to the gradual decline in the plasma B_{12} concentration throughout gestation. Based on gestational week, prevalence of deficiency worldwide may vary from 5% (<28 days gestation) to 72% (immediately prior to delivery) (Koc *et al*., 2006; Ray *et al*., 2008). Vitamin B_{12} deficiency was not reported as a frequent cause of anaemia (Allen, 1997).

Vitamin B_{12} deficiency has been linked to megaloblastic and pernicious anaemia (O"Leary and Samman, 2010) and a decline in cognitive function in older age (O"Leary *et al.*, 2012). High dietary intakes or supplements of folate and vitamin B_{12} decrease elevated plasma homocysteine and reduce the risk of NTD (Wartanowics *et al.*, 2001; Thompson *et al.*, 2009). Anaemia, myelopathy and neuropathy are the main clinical manifestations of vitamin B_{12} deficiency (Charmel, 2006). Increasing folic acid and vitamin B_{12} may reduce megaloblastic anaemia in mothers (West *et al.*, 1999).

2.7 Folate and pregnancy

Folic acid (pteroylglutamic acid) is a yellow, crystalline, water soluble substance with molecular weight 441. It is the parent compound of a large family of folate compounds.

Synthetic folic acid is comprised of 3 parts: pteridine ring, para-aminobenzoic acid and L-glutamic acid (Hoffbrand and Green, 2005) all of which are needed for vitamin activity. Folic acid may also be referred to as pteroglutamate or pteroyl monoglutamate.

Folate is a generic term applied to dietary sources of related components that are involved in the metabolism of nucleic and amino acids, and hence the synthesis of DNA, RNA, and proteins. Folate has a role in recycling homocysteine to methionine. The term folate is broad term referring to both natural and synthetic forms of the vitamin. However, officially the term folate should only be used when the B vitamin is naturally present in food sources. The folic acid should be used when referring to supplement and fortified food sources containing the synthetic form of the vitamin (Bailey, 2000).

Folate from foods exists mainly in the form of methyl- tetrahydrofolate (methyl-THF). Folate requirements are expressed as dietary folate equivalents (DFE). The term DFE includes folate from food and folic acid. Folic acid is a synthetic form of folate, found in supplements and fortified foods and beverages. It is more bioavailable and more stable than folate from food. Low serum and red cell folate levels in early pregnancy are known to be teratogenic and are associated with NTDs in the infant, megaloblastic anaemia of pregnancy, cervical dysplasia and atherosclerosis in the mother. The risk of NTD is higher in infants of obese women. Folate has a role in reducing and maintaining the level of homocysteine in the blood at an optimal level. Raised levels of homocysteine in pregnancy are associated with complications and adverse outcomes of pregnancy. These may include an increased risk of pre-eclampsia, neural tube defects and other congenital abnormalities, low birthweight and preterm delivery, placental abruption and spontaneous pregnancy loss (Vollset *et al.*, 2000).

2.7.1 Folate metabolism

Metabolically, these two forms of folic acid (synthetic versus natural folates) follow slightly different metabolic pathways. Folic acid lacks coenzyme activity and must first be reduced to the metabolically active form tetrahydrofolate. Dietary folate however, is naturally consumed in a more readily available form that does not need to be reduced. Although foods are a good source of folate, they do not necessarily provide the proportions needed for maternal and foetal health. Bioavailability studies have shown that methyl- hydrofolate when ingested in supplement form is as bioavailable as folic acid and is less likely to mask haematological symptoms of vitamin B_{12} deficiency (Pietrzik *et al.*, 2010).

Folate plays a key role in several metabolic pathways during pregnancy including those leading to DNA and RNA synthesis (Patterson, 2008). Folate acts as a coenzyme, facilitating the transfer of carbon to nucleic and amino acids. During periods of rapid cell division that is

growth of the foetus and expanding organs in pregnancy, folic acid requirements increase. This is because more folate is needed to facilitate the carbon transfer reactions and support the rapid periods of cell division that are taking place (Tamura and Picciano, 2006).

During pregnancy, many coenzymes rely on folate to support their function. For example, folate containing coenzymes are needed for the conversion of homocysteine into the amino acid methionine. Research suggests that increased plasma levels of homocysteine (particularly in the third trimester of pregnancy) may be a predictor of low birth weight deliveries (Takimoto *et al.*, 2007).

Homocysteine levels have also been found to be higher in women with pre- eclampsia, but the role folate may play is yet to be firmly established (Mignini *et al.*, 2005).

Folates are found in a wide array of foods. Yeast extract, fortified beverages and green leafy vegetables are all rich sources. Folate is also abundant in organ meats such as liver and nuts, but these are generally not recommended for consumption during pregnancy. In the United States, cereals were found to significantly contribute daily intakes amongst the 68% women who did not take supplements (Tinker *et al.*, 2010).

Lower amounts are also found in vegetable sources. Generally, it can be difficult for women to consume enough folate from their diet, which is why supplementation with 400ug folic acid is recommended before conception and during the first 12 weeks of pregnancy. The level beyond which the risk of delivery a child with neural tube defects significantly reduces is having blood cell folate levels that is greater than (>906nmol/L). Therefore, taking higher dose folic acid supplement 800ug per day will help reach the target blood cell folate levels within an average of 4.2weeks (Bramswig *et al.*, 2009). A woman preparing for pregnancy will need to start taking folic acid supplement for more than one month before conception to improve their folic acid levels.

Although folate can be obtained from food sources, it has low bioavailability that is only small proportions are absorbed and used for metabolic processes. For this reason, women planning to have a baby should always take a folic acid supplement.

2.7.2 Folate deficiency; signs and symptoms

Folate deficiency is a low level of folic acid and derivatives in the body. Also known as vitamin B9, folate is involved in adenosine, guanine and thymidine synthesis (part of DNA synthesis). Signs of folate deficiency are often subtle. Anemia is a late finding in folate deficiency and folate deficiency anemia is the term given for this medical condition. It is characterized by the appearance of large-sized, abnormal red blood cells (megaloblasts), which form when there are inadequate stores of folic acid within the body (Tamparo, 2011).

Additional signs are weakness, sore tongue, headaches, heart palpitations, irritability, and behavioral disorders (Haslam and Probert, 1998). In adults, anaemia (macrocytic, megaloblastic anaemia) can be a sign of advanced folate deficiency. A lack of folic acid can lead to grave symptoms, such as anaemia, depression, stomach and /or intestinal problems, loss of appetite and weight loss. The most serious consequences of a folic acid deficiency can be seen during pregnancy. Here it can lead to abortions, malformations, premature births, heart defects, and development disorders (Scholl and Johnson, 2000).

Women with folate deficiency who become pregnant are more likely to give birth to low birth weight premature infants, and infants with neural tube defects. In infants and children, folate deficiency can lead to failure to thrive or slow growth rate, diarrhoea, oral ulcers, megaloblastic anaemia, neurological deterioration. Microcephaly, irritability, developmental delay, seizures, blindness and cerebellar ataxia can also be observed.

2.7.3 Neural tube defects

Include anencephaly, spina bifida and encephalocele (hernia of part of the brain). The neural tube is a structure that develops into the spinal column and brain during the first 28 days of pregnancy. When the neural tube does not close, this is thought to be a cause of neural tube defects.

A large case- control study undertaken in Norway found that supplementation with at least 400ug/day folic acid may reduce the risk of cleft lip (with or without cleft palate) by about a third (Wilcox *et al.*, 2007).

No adverse effects have been reported with the high consumption of natural folates most concerns relate to the safety of high intakes of folic acid particularly in relation to recognizing symptoms of cobalamin deficiency (Carmel, 2009). For women planning a pregnancy or in the early phases of their pregnancy, (the first 12weeks), 400ug/day folic acid is recommended. For parents that have a neural tube defect themselves, or previous child with neural tube defect, up to 5mg/day folic acid is recommended. Women with diabetes are also recommended to take 5mg/day folic acid before conception and 12weeks after.

For women, folate reserves are only small. Around 10mg is stored in the liver, which is usually used within 2-3 months (Roach and Benyon, 2003). Although there do not appear to be any formal guidelines advising women to take folic acid 3 months prior to pregnancy, from a storage perspective, women would benefit as this would help to top up their liver reserves before proceeding to pregnancy. With regard to rates of folate turnover in pregnancy, scientists have observed that this increases in pregnancy reaching a peak in the 3rd trimester- when foetal growth is highest (Higgins *et al.*, 2000). It is well established that

folate deficiency in early pregnancy can lead to development of neural tube defects but women may also experience megaloblastic anaemia- the formation of large immature cells in pregnancy, mostly in cells that divide rapidly that is red blood cells (Chandra, 2010). Overall, it is important that the body adjusts metabolically throughout the pregnancy to ensure the foetus grows and develops appropriately and has enough energy and nutrients stores for the first few months of life.

An inadequate dietary folate intake results in a reduction of DNA biosynthesis and thereby of cell division, leading to anemia, leucopenia and thrombocytopenia (WHO, 2004a). Moreover, as an effective scavenger of oxidizing free radicals, folic acid acts as antioxidant and can protect bio-constituents such as cellular membranes or DNA from free radical damage (Joshi *et al.*, 2001).

Folate plays a crucial role in the one-carbon metabolism for physiological nucleic acid synthesis and cell division, regulation of gene expression, amino acid metabolism and neurotransmitter synthesis (Djukic, 2007). During pregnancy, increased folate intake is required for rapid cell proliferation and tissue growth of the uterus and the placenta, growth of the fetus and expansion of the maternal blood volume (Rondo and Tomkins 2000). Folate requirements are 5- to 10-fold higher in pregnant than in non-pregnant women (Anthony, 2007), therefore pregnant women may be at risk for folate deficiency. The importance of adequate periconceptional folate supply is well recognized in human health; the link between maternal folate status and foetal neural tube defects (Czeizel and Dudas, 1992) and other congenital malformations (Botto *et al.*, 2002) is generally accepted.

Higher maternal erythrocyte folate concentrations at 28 weeks were associated with higher fat mass and per cent body fat in the offspring (Yajnik *et al.*, 2008). In most countries, women are advised to use folic acid supplements in the periconceptional period: 0.4mg per day when planning a pregnancy, or 4mg per day when a previous pregnancy was affected by neural tube defects (Pitkin, 2007).

Folate deficiencies and vitamin B_{12} deficiencies has been defined as contributing causes of nutritional anaemia (van den Broek, 2003). Karaoglu *et al.*, (2010) recorded higher folate deficiency than iron deficiency in pregnancy. Prior to nationwide mandatory folate fortification programs, folate deficiency was the second most common cause of anemia during pregnancy (Sifakis and Pharmakides, 2000). The prevalence of folate deficiency in pregnancy varies from 1%to 50%, and is higher in economically deprived regions of the

world. Numerous studies illustrate that the prevalence of both folic acid and cobalamin deficiency increase with advancing gestation (Ackurt *et al.,* 1995).

Anaemia due to folate and vitamin B_{12} deficiency is relatively uncommon worldwide but can be best detected by elevated mean cell volume (MCV). Most pregnant women with folate or vitamin B_{12} deficiency do not exhibit macrocytosis (Frenkel and Yardley, 2000; Karaoglu *et al.,* 2010) which may be masked by iron deficiency anaemia. It is important to note that MCV is raised in folate and vitamin B_{12} deficiency, so high MCV may be a more sensitive measure of deficiency of these vitamins in populations where iron deficiency is less prevalent (WHO, 2001).

The association between maternal folate status and fetal neural tube defects (NTDs) is well recognized, as demonstrated by several interventional trials and observational studies (de Bree *et al.*, 1997; Pitkin, 2007). Neural tube develops into the spine and NTDs occur when the brain and skull and/or the spinal cord and the protective spinal column do not develop properly within the first 4 weeks after conception. Folate functions as a co-enzyme in single-carbon transfers in the metabolism of aminoacids and nucleic acids. Moreover, folate is the substrate donor in the remethylation of homocysteine into methionine, catalyzed by methionine synthase and 5, 10-methylenetetrahydrofolatereductase (MTHFR). Altered homocysteine metabolism leading to HHCY has been proposed as the mechanism involved in NTDs given that higher total homocysteine (tHcy) levels were found in plasma or amniotic fluid of NTD infants and their mothers with respect to non-NTD individuals (Locksmith and Duff, 1998; Tamura and Picciano, 2006).

However, the effect of folate supplementation throughout pregnancy on several other outcomes is highly controversial (Fekete *et al.,* 2010). Numerous observational studies suggest a potential benefit of good maternal folate status on birth weight, placental weight or length of gestation (Goldenberg *et al.*, 1992; Frelut *et al.*, 1995; Relton *et al.*, 2005).

2.7.4 Causes of folate deficiency

A deficiency of folate can occur when the body's need for folate is increased, when dietary intake or absorption of folate is inadequate, or when the body excretes (or loses) more folate than usual. Medications that interfere with the body's ability to use folate may also increase the need for this vitamin (Cravo *et al.,* 1996, Pietrzik and Thorand, 1997, Kelly, 1998). Some research indicates that exposure to ultraviolet light, including the use of tanning beds, can lead to a folate deficiency (Borradale *et al.,* 2014). The deficiency is more common in pregnant women, infants, children, and adolescents. Additionally, a defect in homocysteine methyltransferase or a deficiency of $B\text{-}_{12}$ may lead to a so-called "methyl-trap" of

tetrahydrofolate (THF), in which THF is converted to a reservoir of methyl-THF which thereafter has no way of being metabolized, and serves as a sink of THF that causes a subsequent deficiency in folate (Hoffbrand and Weir, 2001). Thus, a deficiency in B_{-12} can generate a large pool of methyl-THF that is unable to undergo reactions and will mimic folate deficiency.

Folate (pteroylmonoglutamate) is absorbed throughout the small intestine, though mainly in the jejenum, binding to specific receptor proteins. Diffuse inflammatory or degenerative diseases of the small intestine, such as Crohn's disease, coeliac disease, chronic enteritis or entero-enteric fistulae, may reduce the activity of pteroyl polyglutamase (PPGH), a specific hydrolase required for folate absorption, and thereby leading to folate deficiency.

2.7.5 Prevention of folate deficiency

Folate is found in leafy green vegetables. Multi-vitamins also tend to include folate as well as many other B vitamins. B vitamins, such as folate, are water-soluble and excess is excreted in the urine.

Most foods (green-leafy vegetables, fruits, liver, bread) contain folates. The highest concentrations are found in liver and yeast (greater than 200microgramme/100gramme), spinach, other greens and nuts. The total folate content of an average Western diet is about 250 microgramme daily, but the amount varies widely according to the type of food eaten and the method of cooking. Folate is easily destroyed by heating, particularly in large volumes of water, 90 percent may be lost (Hoffbrand and Green, 2005). When cooking, use of steaming, a food steamer, or a microwave oven can help keep more folate content in the cooked foods, thus helping to prevent folate deficiency (McKillop *et al.*, 2002).

Folate deficiency during human pregnancy has been associated with an increased risk of infant neural tube defects (Czeizel *et al.*, 2013). Such deficiency during the first four weeks of gestation can result in structural and developmental problems.

Folate deficiency during gestation or infancy due to development by the fetus or infant of autoantibodies to the folate receptor might result in various developmental disorders including autism spectrum disorders (Desai *et al.*, 2016).

Studies suggest that insufficient folate and vitamin B_{12} status may contribute to major depressive disorder and that supplementation might be useful in this condition (Coppen *et al.*, 2005). The role of vitamin B_{12} and folate in depression is due to their role in transmethylation reactions, which are crucial for the formation of neurotransmitters (for example, serotonin, epinephrine, nicotinamides, purines, phospholipids) (Coppen *et al.*, 2005; Karakuta *et al.*, 2009). The proposed mechanism, is that low levels of folate or vitamin B_{12} can disrupt

transmethylation reaction, leading to an accumulation of homocysteine (hyperhomocysteinemia) and to impaired metabolism of neurotransmitters (especially the hydroxylation of dopamine and serotonin from tyrosine and tryptophan), phospholipids, myelin, and receptors. High homocysteine levels in the blood can lead to vascular injuries by oxidative mechanisms which can contribute to cerebral dysfunction. All of these can lead to the development of various disorders, including depression (Coppen *et al.*, 2005; Karakuta *et al.*, 2009).

2.7.6 Some situations that can increase the need for folate

Some situations can increase the need for folate such as haemorrhage, kidney dialysis, liver disease, malabsorption (including celiac disease and fructose malabsorption), tobacco smoking, alcohol consumption, and of course, pregnancy and lactation. Also, some medications can interfere with folate utilization. Drugs such as anticonvulsants, metformin (sometimes prescribed to control blood sugar in type 2 diabetes, birth control pills, and methotrexate (an anti-cancer drug also used to control inflammation associated with Crohn's disease, ulcerative colitis and rheumatoid arthritis). Also, some drugs increase the risk of NTDs and this may be in part because they are folate antagonists (Pimentel, 2000). These drugs include anti-epileptic or anti-convulsant drugs (such as carbamazepine and valproate), insulin, infertility treatment (such as clomiphene), vitamin Analogues (used for acne treatment) and some anti tumour agents such as when methotrexate is prescribed; folic acid supplements are sometimes given with the methotrexate. Methotrexate inhibits cell division and is particularly toxic to fast dividing cells, such as rapidly dividing cancer cells and the progenitor cells of the immune system. Folate supplementation is beneficial in patients being treated with long-term, low-dose methotrexate for inflammatory conditions, such as rheumatoid arthritis (RA) or psoriasis, to avoid macrocytic anemia caused by folate deficiency.

2.7.7 Relationship between vitamin B_{12} and folic acid

Vitamin B_{12} and folic acid (also folate or vitamin B9) are very closely connected in the metabolism. Both play an important role together in methionine synthase; the conversion of dangerous homocysteine to methionine. Folic acid, also known as vitamin B9 belongs to the vitamin B complex. The vitamins of the B complex all work very closely together. However, vitamin B_{12}, folic acid and vitamin B6 work particularly closely in tandem, all playing a role in the deconstruction of homocysteine.

Vitamin B_{12} is responsible for reactivating folic acid, by converting it through various reactions back into tetrahydrofolate, the form of folic acid which the body can use. A vitamin B_{12} deficiency thus leads to an indirect folic acid deficiency: even if enough folic acid is provided for the body, it cannot be used unless a supply of vitamin B_{12} is present; it simply sits in its inactive form. On the other hand, vitamin B_{12} cannot fulfill its role in the deconstruction of homocysteine without folic acid – the two vitamins are dependent on one another (Greenberg *et al.*, 2011).

The active form of folic acid found in the body is known as tetrahydrofolate (THF). However, the folic acid which circulates in the blood is 5-methyltetrahydrofolate (5-MethylTHF, MTHF). Through the use of vitamin B_{12}, this MTHF plays a sort of methyl group ping pong:

MTHF gives off its methyl group to vitamin B_{12} (cobalamin), which becomes methylcobalamin. At the same time, the MTHF folic acid is converted back into its bioactive form, tetrahydrofolate, through the same reaction. The methylcobalamin then gives off its methyl group in a further step to homocysteine, which is then converted to methionine (Fig. 2.1).

Then the cycle begins again as new, with the newly free cobalamin reacting with the next MTHF molecule, and so on. The result of this is twofold; firstly the dangerous homocysteine is converted into the very important methionine, and secondly the folic acid is regenerated into its bioactive form once more. This reaction plays a key role for many metabolic processes which are very relevant to health and wellbeing in the body.

S-Adenosyl methionine (SAM)

The methionine from this reaction is converted in further steps to S-adenosyl methionine (SAM) – one of the most important things for the production of various neurotransmitters and for DNA methylation. The latter specifies how certain genes in our genome are selected and thus has very profound effects on the body as a whole organism.

In this reaction, SAM gives off its methyl group and thus becomes homocysteine, starting the whole cycle mentioned above again as new (Fig. 2.2).

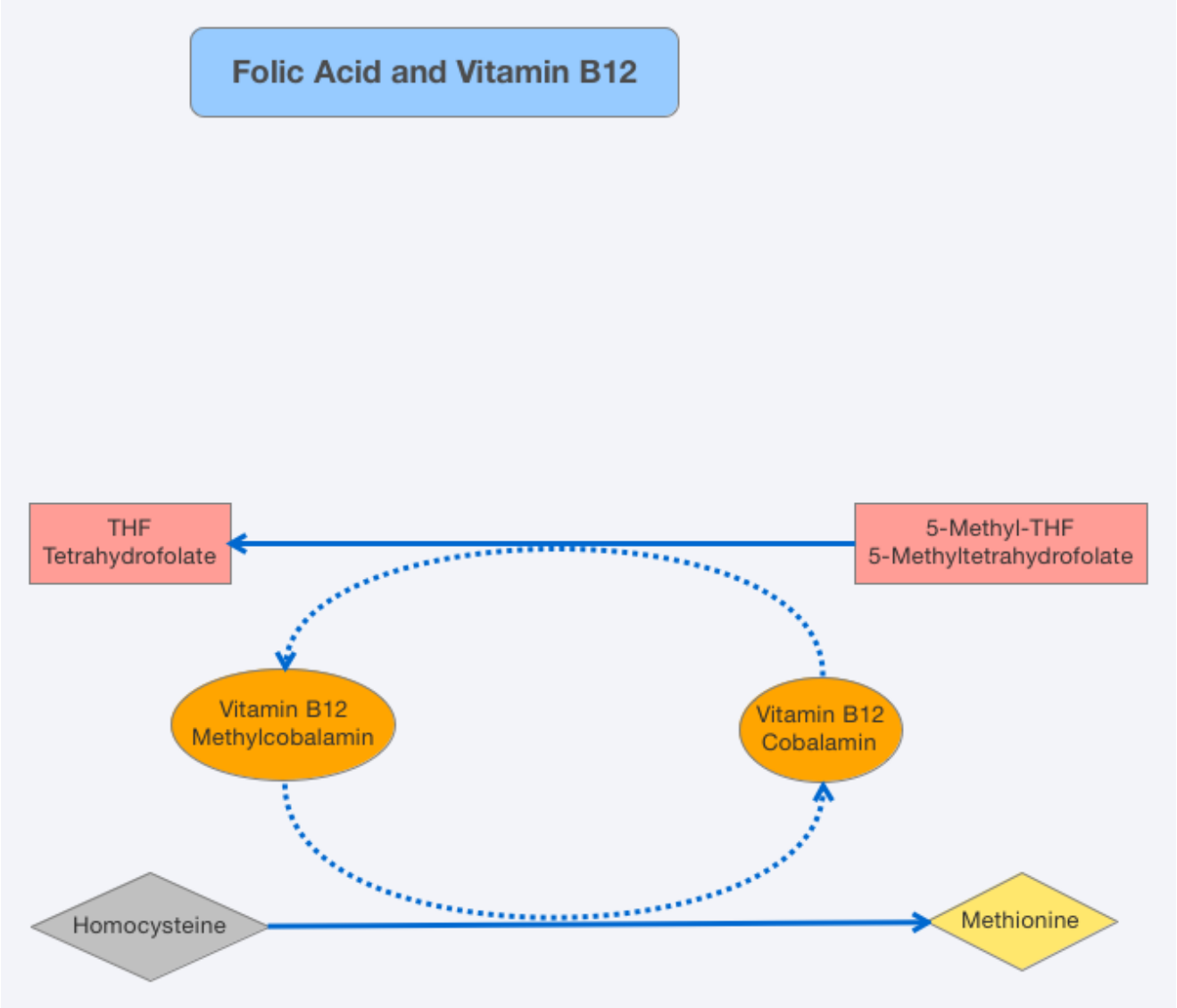

Figure 2.1: Interaction between folic acid and vitamin B_{12} (Scholl and Johnson, 2000).

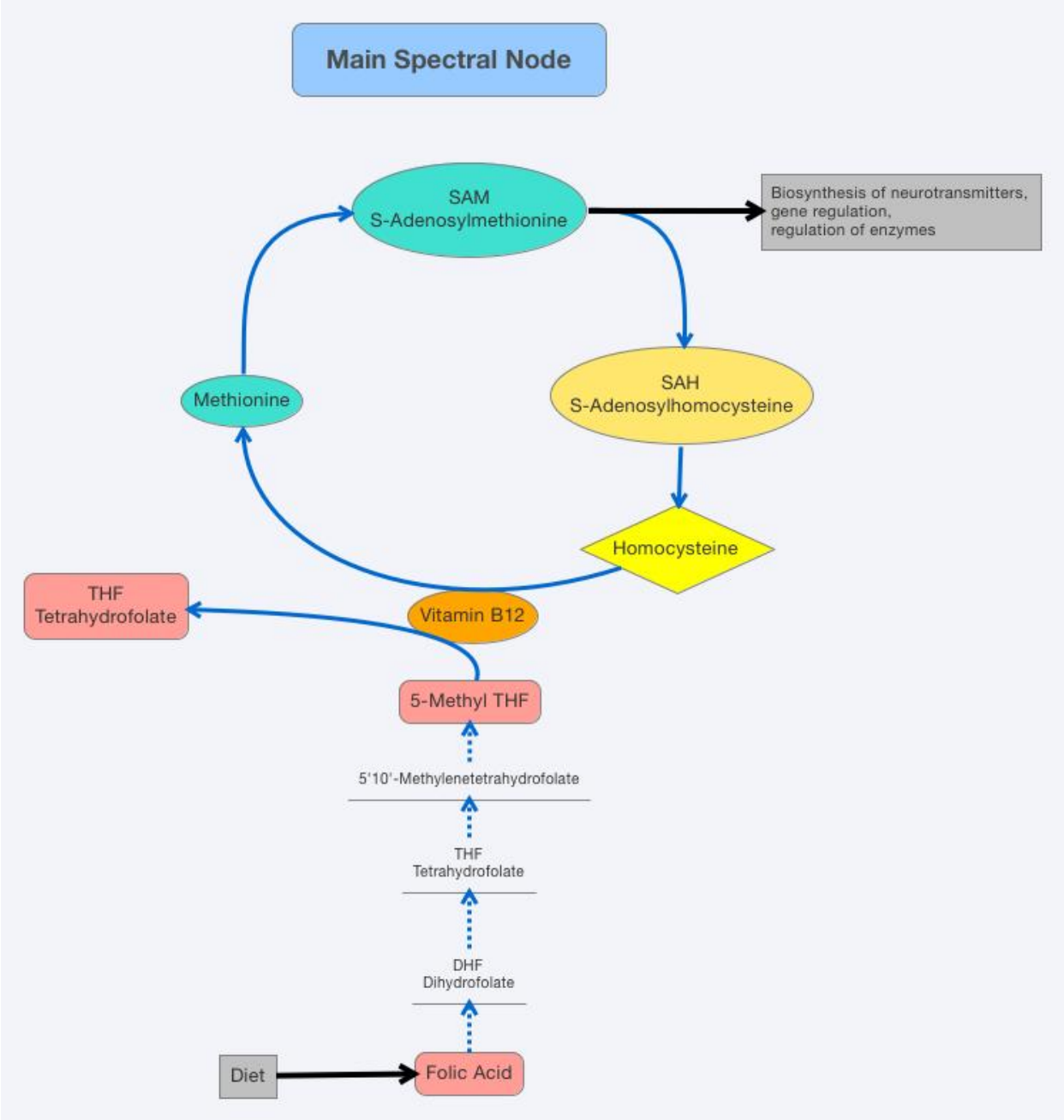

Figure 2.2: Interaction between folic acid and vitamin B_{12} (Scholl and Johnson, 2000).

2.7.8 Folic acid supplementation

The folic acid daily intake requirement almost doubles in pregnancy and is difficult to meet through a normal diet, which is why a folic acid deficiency is especially common here. Gynecologists in most cases recommend taking folic acid supplements of around 400 – 800 µg of folic acid during pregnancy. During breastfeeding, the body's requirement for folic acid remains high – so it can be advisable to take supplements at this time as well (Scholl and Johnson, 2000).

Women who are trying to conceive should be sure to check their folic acid supply before pregnancy starts, because often women who fall pregnant remain unaware for the first two to four weeks, before pregnancy tests available over the counter can confirm this. This period in time, however, is a critical phase for the development of the embryo, during which folic acid is needed urgently. Consequently, folic acid supplies should be optimal from the moment that the decision to try for a child is made. This way, a folic acid store is already being built up, through which a stable folic acid status can be ensured during the pregnancy.

As a result of the close relationship between vitamin B_{12} and folic acid, there are many supplements which combine the two vitamins.

Just like with vitamin B_{12}, there are also a number of active ingredients for folic acid. A promising and very recent development is the direct use of L-5-methyltetrahydrofolate (also L-5-methylTHF, L-methylfolate, MTHF and 5-methylTHF) in food supplements. This is the form of folate which is transported in the blood and which works together with vitamin B_{12} (Greenberg *et al.,* 2011).

The direct use of L-5 MTHF makes sense, since the other synthetic forms of folic acid must first be converted by the small intestine in a multilevel process before they form 5-methylTHF. The final step of this process, in which 5,10-methyltetrahydofolate is converted into L-5-methylTHF, is particularly difficult as a result of impairment caused by enzyme disorders in many people, which is why the direct use of MTHF is recommended. A practical dosage for such supplements should be between 200 and 800 µg per dose (Scholl and Johnson, 2000; Greenberg *et al.*, 2011).

2. 8 Haematological changes in normal pregnancy

Normal pregnancy is characterized by many physiological and haematological changes, which may appear to be pathological in the non pregnant state (Harrison, 1966). It is also one of the physiological conditions capable of causing remarkable and dramatic changes in haematological variables. A pregnancy is influenced by many factors, some of which include culture, environment, socioeconomic status, and access to medical care. The haematological

indices also have an impact on pregnancy and its outcome (Yip, 2000). Blood is a special type of connective tissue composed of formed elements in a fluid matrix. Many of the haematological indices are influenced by many factors like sex, seasonal variation, lactation, pregnancy, health and nutritional status (Smith, 1993). In normal pregnancy, the haematological indices of an individual to a large extent reflect their general health (WHO, 2004). Osonuga *et al.*, (2011) and Shaw *et al.*, (2010) have identified the haematological indices of the pregnant women as one of the factors affecting pregnancy.

The haematological profile of pregnant woman has an impact on pregnancy and its outcome (Madan *et al.*, 2006; Akingbola *et al.*, 2006; Bang and Lee, 2009). The most common haematological indices are the indicators of haemoglobin concentration. Low haemoglobin in the blood is widely identified as a haematological abnormality and it is associated with adverse pregnancy outcome (James *et al.*, 2008). It is very difficult to define a normal reference range for haemoglobin concentration during pregnancy. Decrease in PCV values could be due to marked increase in plasma volume associated with normal pregnancy causing dilution of many circulating factors and cells resulting in physiological anaemia (Salawu and Durosinmi, 2000; James *et al.*, 2008; Imoru and Emeribe, 2009). The usual drop in PCV by the late second trimester and thereafter stabilizes in the third trimester, when there is a reduction in maternal plasma volume is due to an increase in the levels of atrial natriuretic peptide (Barriga *et al.*, 1994; Ajzenberg *et al.*, 1998).

The hematologic status in pregnant woman can be evaluated by measuring different blood indices such as haemoglobin concentration, packed cell volume (PCV), total white blood cell (WBC) count and differential count, mean corpuscular volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), and platelet count during each of three trimesters of pregnancy. Red cell mass (driven by an increase in maternal

erythropoietin production) also increases, but relatively less, compared with the increase in plasma volume, the net result being a drop in hemoglobin concentration. Thus, there is dilutional anemia. The drop in hemoglobin is typically by 1–2 g/dL by the late second trimester and stabilizes thereafter in the third trimester, when there is a reduction in maternal plasma volume (owing to an increase in levels of atrial natriuretic peptide) (Barriga *et al.*, 1994; Ajzenberg *et al.*, 1998). Women who take iron supplements have less pronounced changes in hemoglobin, as they increase their red cell mass in a more proportionate manner than those not on hematinic supplements. The red blood cell indices change little in pregnancy.

However, there is a small increase in mean corpuscular volume (MCV), of an average of 4 fl in an iron-replete woman, which reaches a maximum at 30–35 weeks gestation and does not suggest any deficiency of vitamins B_{12} and folate. Increased production of RBCs to meet the demands of pregnancy, reasonably explains why there is an increased MCV (due to a higher proportion of young RBCs which are larger in size).

Mean corpuscular volume (MCV) is an unreliable marker of iron deficiency in pregnancy. Stimulation of erythropoiesis leads to a physiologic increase in MCV during gestation that counterbalances the microcytosis of iron deficiency (van den Broek, 1998). A low MCV, defined as an MCV, 80 fL, is highly sensitive (Guyatt *et al.*, 1992), but not specific, for iron-deficiency anemia. Anemia is the most common hematological problem in pregnancy, followed by thrombocytopenia. Crocker and colleagues postulated that MCV does not change significantly during pregnancy and haemoglobin of 9g/dl in association with a MCV of 84fl probably indicates co-existent of iron deficiency or some other pathology (Crocker *et al.*, 2000).

In 2011, 29% (496 million) of non-pregnant women and 38% (32.4million) of pregnant women aged 15-49 years were anaemic (Stevens *et al.*, 2013). About 20% of maternal deaths occur due to anaemia (Jiji and Rajagopal, 2014). The platelets count is slightly lower in pregnant than in non pregnant women (Abbassi-Ghanavati *et al.*, 2006). Due to hemodilution secondary to expansion of plasma volume, the platelets count in normal pregnancies may decrease by approximately 10%, with most of this decrease occurring during the third trimester (Ballem 1988; Boehlen *et al.*, 2000; McCrae, 2003; Jensen *et al.*, 2011), although the absolute platelets count tends to remain within the normal reference range in most patients (Ballem 1988; Boehlen *et al*, 2000; McCrae, 2003). Platelets counts may be lower in women with twin compared with singleton pregnancies, possibly due to greater thrombin generation (Tsunoda *et al.*, 2002). Leukocytosis is almost always associated with pregnancy (Akinbami *et al.*, 2013), but the full sequential changes of the various cell types responsible for this observed leukocytosis have not been clearly determined in all geographical locations and physiological conditions (Akinbami *et al.*, 2013; Onwukeme and Uguru, 1990).

2.8.1 Anaemia of pregnancy

Physiologic anaemia is the term often used to describe the fall in haemoglobin concentration that occurs during normal pregnancy resulting from plasma volume increase above normal by the end of gestation although the red cell masses itself increase by some and still leads to a fall in haemoglobin concentration with a feature of normocytic and normochromic type of

anaemia (Hoffbrand, 2003). Physiologic anemia of pregnancy reflects an expansion of plasma volume of 50% relative to the increase in the red blood cell (RBC) mass of 25% (Milman *et al.*, 2000).

Two-third of all pregnant and half of all non-pregnant women in Africa have anemia (WHO, 2000). Anemia in pregnancy arises from a variety of factors which include the physiological hemo-dilution of pregnancy, increased demand of the fetus on maternal stores of iron and folic acid, poor nutritional diet, infections and infestation such as malaria and hookworm and some adverse cultural practices (WHO, 2000; 2001).

Anemia of pregnancy is a well-recognized global health problem, affecting almost half of pregnant women (WHO, 2001). The World Health Organization (WHO) defines anemia of pregnancy as hemoglobin, 11 g/dL, or hematocrit, 33%, at any time during the pregnancy (WHO, 2011). The WHO defines severe anemia in all persons as haemoglobin of 7 g/dL and very severe anemia as haemoglobin of 4 g/dl (WHO, 2011). Globally, the most common cause for anemia of pregnancy is iron deficiency, arising from maternal-fetal transfer of iron, frequently aggravated by decreased maternal iron reserves (Lee and Okam, 2011). The Nutrition Impact Model Study, a systematic analysis of 257 population-representative data sources from 107 countries, estimated the global prevalence of anemia in pregnancy at 43% in 1995 and 38% in 2011 (Stevens *et al.*, 2013). Dim *et al*, (2007), reported a 40% prevalence of anaemia in pregnant women in Enugu, South east of Nigeria using a haemoglobin <11g/dl cutoff. Isah *et al*, (1985) reported a prevalence of 37% in Zaria, Nigeria among the elite pregnant women. Anaemia contributes to intrauterine growth restriction, preterm labour, abortions and it is also a primary cause of low immunity of both the mother and the baby, which makes them prone for several life threatening infections (Stevens *et al.*, 2013).

2.8.2 Iron and pregnancy

Iron is essential for many metabolic processes. It shares with other transition metals two properties of particular importance in biology which include the ability to exist in more than one relatively stable oxidation state and the ability to form many complexes (Worwood and Hoffbrand, 2005). Its ability to exist in both ferrous and ferric states underlies its role in critical enzyme reactions concerned with oxygen and electron transport and the cellular production of energy (Worwood and Hoffbrand, 2005). Iron functions as a component of proteins and enzymes.

There are two types of iron in the diet: haem and non-haem iron. Haem iron from meat, poultry and fish is typically 20 to 30 percent absorbed, and absorption is not significantly affected by other components of the diet. Non-haem iron from non-animal sources such as plant foods (lentils and beans), iron medication, and iron fortificants in food is less bioavailable, with absorption of 5% or less. Each day the body absorbs approximately 1-2 milligram (mg) of iron to compensate for the 1-2 mg of iron that the (non-menstruating) body loses (Institute of Medicine, 2001).

Although haem iron is absorbed better than non-haem iron, most dietary iron is non-haem iron (Miret, 2003). Absorption varies with physiological requirements, the iron status of the individual and dietary composition. Absorption of non-haem iron can be promoted by vitamin C and the presence of fish, meat or poultry. Inhibitors of non-haem iron absorption include phytates (in legumes, bran, grains and rice), polyphenols (in tea and coffee, grains and red wine), and vegetable proteins such as those in soybeans. Interestingly, legumes, whole grains and rice are still useful sources of iron, particularly for vegetarians and vegans. The average American diet provides 10-15 milligrams of iron daily in the form of haem and non-haem iron. Other nutritional sources of iron are bread and green vegetables (WHO, 2004c).

2.8.3 Iron requirements in pregnancy

Iron requirements in pregnancy need to provide for the growing foetus and increased maternal blood volume. Foetal requirements tend to be met at the expense of the mother. Although first trimester iron requirements are lower than for the non-pregnant woman, requirements are markedly higher by the third trimester (Hallberg, 2001). Some of the additional requirement is offset by menstrual savings and increased maternal absorption of iron: non haem iron absorption is known to increase in pregnancy, especially in third trimester (Hallberg, 1994).

In a typical pregnancy, maternal iron requirements include 300 to 350 mg for the fetus and the placenta, 500 mg for the expansion of the maternal RBC mass, and 250 mg associated with blood loss during labor and delivery (Scholl, 2011). The requirement for iron increases gradually from 0.8 mg per day in the first trimester to 7.5mg per day in the third (Bothwell, 2000). Yet, the average daily absorption of iron from western diets is only 1 to 5 mg (Lee and Okam, 2011). Therefore, women cannot fulfill their iron needs from normal food intake, and must draw upon iron stores, increasing the risk of iron-deficiency anemia.

Some studies emphasized that tea reduces iron absorption but does not influence iron status in people with adequate iron stores (Dangour *et al.*, 2001; Temme and Van Hoydonck, 2002; Mennen *et al.*, 2007). Meat is a good source of high quality protein, iron and zinc and of all the B-vitamins except folic acid.

Consuming tea between meals and simultaneously consuming vitamin C and/or meat, fish and poultry were the main dietary recommendations to prevent anemia (Zijp *et al.*, 2000). A recent publication reported that green and black tea had the potential risk of diminished folic acid bioavailability (Alemdaroglu *et al.*, 2008).

For women to sustain an adequate iron balance during pregnancy, body iron reserves need to be at least 500mg when a woman conceives (Milman, 2006b). Compared with other aspects of iron metabolism, comparatively, less is known about iron transport to the foetus but has shown that this process is highly regulated (McArdle *et al.*, 2008). Previous papers have reported that the number of transferrin receptors in the placenta can upregulate, as can the number of iron channels; facilitating the transport of iron from the mother to the child (Gambling *et al.*, 2001).

Previous studies have shown that women lose around 17.6ml menstrual blood during each cycle, which is equivalent to 0.43mg iron per day (Harvey *et al.*, 2005). If this is not replenished from dietary sources women may be iron deficient or have low iron stores at the start of pregnancy. Haemoglobin mass also expands rapidly during pregnancy; requiring around 570mg iron and nearly 300mg is lost from the skin and faecal/ urinary excretions. Without including blood lost during delivery, total iron losses during pregnancy total to around 1000mg, exceeding the iron stores of most women, even those in Western regions (Gautam *et al.*, 2008).

In particular, the metabolism of copper and iron are interlinked, although mechanisms of action need to be confirmed. Deficiencies in either of these can alter the distribution of the other mineral (Gambling *et al.*, 2008).

A Chinese study compared the dietary habits of 1189 women in the third trimester who were both healthy and diagnosed with anaemia. It was concluded that low iron intakes, low intakes of iron enhancers and high intake of iron inhibitors all contributed to the development of pregnancy iron deficiency anaemia in the study (Ma *et al.*, 2002). Some disease conditions, especially malaria parasitaemia and pregnancy-induced hypertension (Samuels *et al.*, 1987; Ogbodo *et al.*, 2010) are known to cause an increase in serum iron, which may induce oxidative stress, with associated adverse pregnancy outcomes.

2.8.4 Iron deficiency anaemia

Iron deficiency anaemia (IDA) is a well documented nutritional deficiency during pregnancy in both developed and developing countries (Aikawa *et al.,* 2005; Scholl, 2005) that can exist with or without anaemia. Although iron deficiency is a common cause of anaemia, anaemia may also result from other causes (i.e. deficiencies of folate, vitamin B_{12} and vitamin B_6).

The spectrum of iron deficiency anaemia can be characterized in terms of three stages: iron depletion (low iron stores based on a fall in serum ferritin: normal haemoglobin), iron deficient erythropoiesis (depleted iron stores based on serum ferritin, transferrin saturation and normal haemoglobin) and iron deficiency anaemia (anaemia based on depleted iron stores and low haemoglobin).

Iron deficiency anaemia is when levels of red blood cell production fail to match rates of destruction meaning that haemoglobin levels, amongst other biochemical parameters, become reduced (Cavill *et al.,* 2006). IDA is common among women in their reproductive years in particular if the women are poor, pregnant, and members of an ethnic minority. IDA is the hematologic complication of pregnancy and is associated with increased rates of premature birth, low birth weight and prenatal mortality. IDA is known to be an important factor in maternal death, the poor cognitive development of children and decreased work capacity of the mother.

According to the United Nations (UN) estimates, approximately half of pregnant women suffer from anemia worldwide. Anemia prevalence during pregnancy differed from 18% in developed countries to 75% in South Asia (Wang *et al.,* 2002). Nutritionally related iron deficiency is the main cause of anaemia throughout the world. It is especially common in women of reproductive age and particularly during pregnancy. The demand for iron increases about six to seven times from early pregnancy (Christensen and Ohls, 2004). Besides poor nutrition, frequent labour, multiparity, abortions, parasitic infestations, consuming excess tea or coffee after meals are determined as the predictors of anaemia in reproductive age women. Most iron transfer to the foetus occurs after 30 weeks of gestation which correspond to the time of peak efficiency of maternal iron absorption (Sakande *et al.,* 2004)

Iron deficiency causes impaired red blood cell function and symptoms of weariness, poor concentration and increased risk of infection, and appears to be more common in pregnant women. Anaemia increases the risk of post- partum haemorrhage, infection, mortality, heart failure (Scholl and Reilly, 2000).

Severe maternal iron deficiency can result in a suboptimal iron supply to the foetus, with associated increased risks of foetal death, perinatal mortality, preterm delivery, and low birthweight failure (Scholl and Reilly, 2000). In pregnancy, the body undergoes vast homeostatic changes in order to regulate iron metabolism, preventing both deficiency and overload. However, despite this, iron deficiency anaemia remains a frequent occurrence with as many as 1 in 2 women being diagnosed in pregnancy (Scholl, 2005). The reduction in iron status of the pregnant women predispose the women to adverse pregnancy outcome which is small for gestational age, since anaemia diagnosed before mid-pregnancy has been found to be associated with small for gestational age (Scanlon *et al.*, 2000)

Long term consequences of maternal iron deficiency on the offspring include effects on cognition, behavior, motor development, activity and physical capacity, and may not be reversible. Further, infants of iron deficient mothers are more likely themselves to have low iron stores and be susceptible to iron deficiency (Allen, 1997).

Iron deficiency is more likely with multiple gestation, low socioeconomic status and poor educational attainment, in adolescent women, and in those with a short inter-pregnancy interval. Previous use of oral contraceptives, which limits menstrual flow, tends to result in a favourable iron status. There is a definite need for early assessment of some nutritional parameters, particularly serum iron, as it has been found that iron supplementation started after mid pregnancy may not be able to prevent the consequences of iron-deficiency anaemia (Allen, 2001; Scholl, 2005). Routine assessment of blood parameters, such as serum ferritin in pregnant women, identify those with haemochromatosis who might be at risk of developing clinical effects later in life.

2.8.5 Diagnosis of iron-deficiency anemia

Iron status and body iron can be monitored using several biochemical markers. However, classical indicators of iron status, for example haemoglobin levels, are not necessarily the best markers of iron status. Measuring iron levels is particularly different during pregnancy because inflammation, increased plasma volume resulting in haemodilution (Horton *et al.*, 2013) and erythropoiesis all influence biochemical markers of iron status (Mor, 2008).

2.8.5.1 Haemoglobin level

Almost two-thirds of the iron in the body (approximately 2.5grams of iron) is found in haemoglobin, the protein red blood cells that carries oxygen to tissues, and about 15 percent is in the myoglobin of muscle tissue. Iron is present in haemoglobin as a carrier of oxygen in the blood, the liver, muscle, tissue and many cell enzymes. Maternal haemoglobin declines

progressively during pregnancy due to haemodilution and may be accentuated by iron-deficient erythropoiesis, with a nadir reached at 24 to 32 weeks' gestation (Williams and Wheby, 1992; Milman *et al.*, 2000).

Diagnosing iron deficiency anaemia in pregnancy from haemoglobin concentration can therefore, present some problems due to haemodilution of pregnancy (the plasma volume increases to a greater extent than red blood cell mass) resulting in lower haemoglobin concentrations. Lower haemoglobin concentrations are normal at certain stages of pregnancy rather than indicating iron deficiency anaemia. According to Center for Disease Control (CDC), a haemoglobin concentration of less than 11.0g/dl in the first and third trimesters and less than 10.5g/dl in the second trimester is indicative of anaemia (WHO, 2001). High haemoglobin concentrations, which reflect inadequate plasma volume expansion, are associated with adverse pregnancy outcome so there is a Ushaped relationship between haemoglobin concentration and favourable pregnancy outcome. Due to considerable variation in haemoglobin level, it cannot be used as a single parameter to estimate iron status.

2.8.5.2 Serum iron

Iron requirements in pregnancy need to provide for the growing foetus and increased maternal blood volume. Foetal requirements tend to be met at the expense of the mother. However, levels during infection could be misleadingly higher, which can cause under diagnosis of iron deficiency. Iron is present in haemoglobin as a carrier of oxygen in the blood, the liver, muscle, tissue and many cell enzymes. Over 60% of iron is in haemoglobin, and about 25% as ferritin iron stores, mainly in the liver. The serum iron reflects both iron recycling from macrophages and iron absorbed from the diet. It demonstrates diurnal variation, with a rise in the morning and fall at night (Tietz *et al.*, 1994). Serum iron is also influenced by recently ingested meals. Therefore, no single value is diagnostic of iron deficiency (Auerbach and Adamson, 2016). Serum iron should be drawn after an overnight fast.

2.8.5.3 Transferrin

Serum iron circulates bound to its transport protein, transferrin. Transporting iron from one organ to another is accomplished by the reversible binding of iron to the transport protein, transferrin, which will then form a complex with a highly specific transferrin receptor (TfR) located on the plasma membrane surfaces of cells. Intracellular iron availability is regulated through the increased expression of cellular TfR concentration by iron-deficient cells. Total iron-binding capacity (TIBC) and transferrin are measurements of iron transport proteins that

increase in iron deficiency. Inflammation, chronic infection, malignancies, liver disease, nephrotic syndrome, and malnutrition can lower TIBC, whereas pregnancy can raise it, in the absence of iron deficiency (van den Broek, 1998).

2.8.5.4 Plasma transferrin saturation

Plasma transferrin saturation is the ratio of plasma iron to transferrin. A saturation of 15% suggests an inadequate supply of iron, (Camaschella, 2015) either because of low total body iron (iron deficiency) or due to trapping of iron in macrophages (anemia of inflammation).

2.8.5.5 Soluble transferrin receptor

The soluble transferrin receptor (sTfR) is a truncated fragment of the membrane receptor. In iron deficiency, synthesis of transferrin receptors, and sTfR, is increased (Punnonen *et al.*, 1997). Serum transferrin receptors (sTfR) are also highly regarded biomarkers – when supplies of iron are inadequate. Serum transferrin receptors (sTfR) upregulate to take up higher levels of transferrin-bound iron. Once used, sTfR are shed: therefore, elevated levels are a good indicator of iron deficiency anaemia (Rusia *et al.*, 1999). Unlike TIBC and ferritin, sTfR concentrations are not affected by inflammation (Mast *et al.*, 1998). A meta-analysis of 10 studies of sTfR showed that the assay had a sensitivity of 86% and a specificity of 75% (Infusino *et al.*, 2012). However, the assay is not standardized and is not used in routine diagnosis of iron-deficiency anemia.

In summary, haemoglobin, the percentage of transferrin saturation and plasma ferritin are adequate to assess iron status in the majority of pregnant women, and the combination of anemia and ferritin, 15 to 30 ng/ml is diagnostic of iron deficiency (WHO, 2001).

2.8.5.6 Serum ferritin

Ferritin reflects total body iron stores. Ferritin is the major iron-storage protein found not only in the spleen, liver and bone marrow but also in the mucosal cells of the small intestine, in the placenta, kidneys, testes, skeletal muscles and in the circulating plasma (Crichton, 1973). Ferritin provides iron for the synthesis of iron-containing proteins including haemoglobin and myoglobin. Its concentration declines early in the development of iron deficiency and is highly correlated with bone marrow iron stores, and is decreased before changes in transferrin saturation, serum iron, or haemoglobin concentration occur so that its measurement is superior to the measurement of transferrin saturation or serum iron concentration (Lipschitz *et al.*, 1974, Puolakka *et al.*, 1980, Kaneshige, 1981) and erythrocyte protoporphyrin values (Romslo *et al.*, 1983) in the diagnosis of iron deficiency. Ferritin is

also an acute-phase protein; acute and chronic diseases can result in increased ferritin concentration, potentially masking an iron-deficiency diagnosis. Ferritin concentration declines gradually in pregnancy and usually falls markedly between 12 and 25 weeks of gestation, probably as a result of iron utilization for expansion of the maternal red blood cell mass (Idjradinata and Pollitt, 1993). Ferritin levels are considered the gold standard for the diagnosis of iron-deficiency anemia in pregnancy (Clark, 2009).

Iron deficiency is the only clinical situation associated with extremely low values of ferritin. In pregnancy, serum ferritin concentration is maximum at 12-16 weeks gestation, and then falls with advancing gestation to reach a nadir during third trimester (Puolakka *et al.*, 1980; Kaneshige, 1981; Romslo *et al.*, 1983) and increases during the month before delivery. The nadir is about 15 ng/ml without iron supplementation and 20ng/mL with it (Milman *et al.*, 1991). Prenatal mineral and vitamin supplement given from the first trimester would maintain serum ferritin at a higher concentration (Puolakka *et al.*, 1980; Milman *et al.*, 1994; Scholl *et al.*, 1997). In the absence of active comorbidity, ferritin values 100ng/ml indicate adequate iron stores and a low likelihood of iron-deficiency anemia (Guyatt *et al.*, 1992). Previous report indicates that virtually all patients with serum ferritin concentrations less than 15 η g/mL are iron deficient, with a sensitivity and specificity of 59% and 99%, respectively (Guyatt *et al*., 1992). A cutoff limit of 30 η g/ml may increase its sensitivity to 92% (Romslo *et al.*, 1983; Mast *et al.*, 1998).

The generally accepted cut-off level for serum ferritin below which iron stores are considered to be depleted is 15 ng/mL for people aged 5 years and older and 12 ng/mL for people younger than 5 years of age (WHO, 2001). A range of cut- off values in the past have been used to define iron deficiency in pregnancy but 30ug/L or less is thought to be most accurate (van den Broek *et al.*, 1998).

It is important however to consider that single parameters are of limited value and combinations of different biomarkers of iron status are more accurate for the diagnosis of iron deficiency anaemia (van den Broek *et al.*, 1998). Serum ferritin (a measure of iron stores), transferrin receptors (the degree of iron deficiency after depletion of stores) and haemoglobin measurements (measure whether anaemia is present) may be one of the most accurate ways to determine iron status and to ensure that the entire spectrum of iron deficiency is covered. Other red cell indices such as zinc protoporphyrin, mean cell volume (MCV), serum iron saturation, total iron binding capacity (TIBC), transferrin saturation, serum transferrin and haptoglobin can also be used and calculated (Wish, 2006, Zimmermann, 2008). Ideally the

best indicator of iron deficiency is a combination of at least 3 tests with abnormal values for at least 2 indicating suboptimal iron status (van den Broek *et al.*, 1998).

2.8.6 Iron supplementation in pregnancy

This is recommended generally during pregnancy when requirements are higher and may not be met through the diet alone. Historically for the treatment of iron deficiency anaemia, high dose of iron supplement has been prescribed, often up to 300mg ferrous iron /day (Cook, 2005).

More recently studies have shown that lower dose iron supplementation containing in the region of 30mg/day can protect against iron deficiency anaemia in pregnancy and infancy without yielding unfavourable side effects (Rioux and LeBlanc, 2007). Milman *et al.*, 2005, Zhou *et al.*, 2009 have shown that supplementation with 40mg/day from mid-pregnancy to 8weeks after birth prevents iron deficiency anaemia without causing gastrointestinal symptoms associated with higher intakes. It is recommended that iron supplements are taken between meals or at bedtime to promote absorption (Milman, 2006a). The CDC recommends that all pregnant women begin a 30 mg per day iron supplement at the first prenatal visit, while WHO suggests 60 mg per day for all pregnant women, (WHO, 2001) whereas British guidelines do not recommend any routine iron supplementation in pregnancy (Pavord *et al.*, 2012).

Some women experience side effects in response to iron medications during pregnancy, such as constipation, and nausea, increased oxidative stress and risk of gestational diabetes in pregnancy (Afkhami-Ardekani and Rashidi, 2009). Absorption of iron medications is best on an empty stomach, but this may be associated with more side effects. Low dose iron medications are associated with fewer side effects, and ferrous gluconate (for example, Fergon) appears to be less irritating (Yip, 1996). In addition, compliance to iron supplement in pregnancy is generally inadequate with only 49.7% women taking supplements in the 2nd and 3rd trimesters (Habib *et al.*, 2009).

Iron medication should not be given to women with haemochromatosis (Heath and Fairweather-Tait, 2003), a recessively inherited disease that results in iron overload. The clinical effects of haemochromatosis, because of deposition of iron in the liver, heart, and pancreas, are not usually manifest or of concern in women of child bearing age because of menstrual losses help to maintain iron balance.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Area of the study

This is a study carried out at the University of Calabar Teaching Hospital (UCTH), Calabar, Cross River State in the southern part of Nigeria. It is a 600-bed tertiary health facility established in 1979, though has been in existence since 1887 as St. Margaret Hospital. UCTH is a referral hospital located in the heart of the town where people of all socio-economic background assess healthcare. Calabar also referred to "Canaan City" is the capital of Cross River State and has often been described as the tourism capital of Nigeria. Administratively, the city is divided into Calabar Municipal and Calabar South Local Government Areas. It has an area of 406 square kilometres (157 sqmi) and had a population of 371,022 at the 2006 census, but presently having up to 4 million population.

3.2 Study population

Pregnant women who visited the Antenatal Care Clinic (ANC) of the University of Calabar Teaching Hospital (UCTH), who were in their first trimester of pregnancy during the data collection period (February-October 2016) and willing to participate were recruited into this study. Non pregnant, age and parity matched apparently healthy women were also recruited as controls.

3.3 Study design

The study was a longitudinal study involving pregnant women who were followed up during the course of their pregnancy from the 1st to the 3rd trimester.

3.4 Subjects selection

Women who were screened and certified pregnant and who were in the first trimester (10 weeks gestation) of the present pregnancy were recruited applying or using the non-probability sampling technique. All those who met the set inclusion criteria were recruited into the study. Questionnaires were administered to the women to determine demographic characterization.

Information was obtained on age, educational level attained, parity, home size and income earnings.

3.4.1 Inclusion criteria

The inclusion criteria for the study group are as follows;

- Normal pregnant women free from hypertension, diabetes mellitus, chronic renal disease, sickle cell anaemia, and human immunodeficiency virus (HIV) infection.
- Pregnant women attending antenatal care in UCTH, not taking therapeutic diets and supplements other than iron, folic acid, vitamin C and calcium supplements.

3.4.2 Exclusion criteria

Pregnant women who presented with diabetes mellitus, chronic renal disease, sickle cell anaemia, hypertension and human immunodeficiency syndrome (HIV) infection were excluded.

3.5 Ethical clearance

Ethical clearance was obtained from the Health Research Ethical Committee (HREC) of the University of Calabar Teaching Hospital**,** UCTH, Calabar (appendix III). The informed written consent was also signed by each participant (appendix I).

3.6 Sample size determination

Minimum sample size was calculated using Singh and Masuku (2014) derived from Yaro Yamane formular.

$n = N/1+Ne^2$

Where;

n = minimum sample size

N = population size of the pregnant women in the 1^{st} trimester

e = alpha level = 0.05

$n = 100/1+100\ (0.05)^2$

$= 100/1+100\ (0.0025)$

Minimum sample size = 80

The calculated sample size of 80 was taken as the minimum required for answering all the objectives.

Seventy-two (72) pregnant women in their first trimester (10 weeks gestation) were enrolled for the study and compared with fifty (50) age/sex matched apparently healthy non pregnant women as control group.

3.7 Sample collection

Five (5) milliliters of venous blood was collected from each participant in the 10 weeks gestational period representing 1st trimester collection at the clinic. Two (2) milliliters of the blood was transferred into ethylene diamine tetra-acetic acid (EDTA) bottle at a concentration of 1.5mg/ml of blood for the analysis of full blood count. The remaining 3 milliliters was dispensed into a plain sample bottle. The same procedure was repeated in the 24 and the 36 weeks gestation representing 2nd and 3rd trimesters sample collections respectively. The blood samples were centrifuged after allowing for 30 minutes for clotting and retraction at room temperature. Serum was then collected, transferred to double aliquots for total protein, albumin, serum iron and unsaturated iron binding capacity, ferritin, folate, serum transferrin receptor, vitamin $B_{12.}$ The serum samples were stored in the freezer (-20^{oC}) until analyzed spectrophotometrically for serum iron, unsaturated iron binding capacity, total protein and albumin and by enzyme linked immunosorbent assay (ELISA) methods for ferritin, serum transferrin receptors, folate and vitamin B_{12}. Total iron binding capacity and transferrin saturation were calculated. The weight and height of the pregnant women were measured and the body mass index was calculated during their visits at the antenatal care clinic.

3.8 Laboratory analysis

3.8.1 Full blood count (FBC)

Full blood count was assayed using HaematologyAutoanalyser Sysmex KX-21N.

Principle: Blood sample collected into EDTA bottle is aspirated, measured to a predetermined volume, diluted at the specified ratio, and then fed into each transducer. The transducer chamber has a minute hole called the aperture. On both sides of the aperture, there are the electrodes between which flows direct current. Blood cells suspended in the diluted sample pass through the aperture, causing direct current resistance to change between the electrodes. As direct current resistance changes, the blood cell size is detected as electric pulses.

It directly measures the white blood cell count (WBC), red blood cell count (RBC), haemoglobin (Hb), haematocrit (PCV), platelet (PLT), absolute lymphocyte (LYM), absolute

mixed count (MXD), and absolute neutrophil (NEUT) count while the remaining parameters are calculated or derived: mean cell volume (MCV), mean cell haemoglobin (MCH), and mean cell haemoglobin concentration (MCHC).

Procedure:

Whole blood mode (50 µL sample volume)

The Liquid crystal display (LCD), display "WB" and "READY" on the screen.
The sample identifier was entered by pressing sample number. Using the numeric keys, the sample identifier or number was entered and then pressed **[ENTER]**. The specimen tube being well mixed, the stopper removed, and the tube held up to the sample probe the start switch was then pressed. When the KX-21N beeps twice, the sample was removed from the sample probe. Results were printed on the thermal printer after 60 seconds.

Biochemical indicators

Serum ferritin, serum transferrin receptors, folate, total protein, albumin, serum iron, unsaturated iron binding capacity, and vitamin B_{12} were analyzed in this study. Two others, total iron binding capacity (the sum total of iron and unsaturated iron binding capacity) and transferrin saturation (the ratio of iron to TIBC) were calculated. These particular biochemical indicators were chosen because they are typically used to assess nutritional status.

3.8.2 Folate determination using enzyme linked immunosorbent assay (ELISA) kit by Calbiotech.

Principle: Upon mixing the biotinylated antibody with a serum containing the antigen, a reaction results between the antigen and the antibody. After a short incubation, the enzyme conjugate is added which permits an increase in sensitivity for low concentrated samples. Upon the addition of the enzyme conjugate, competition reaction results between the enzyme analog and the antigen in the sample for a limited number of antibody binding sites. A simultaneous reaction between the biotin attached to the antibody and the streptovadin immobilized on the microwell occurs. This effects the separation of the antibody bound fraction after decantation or aspiration. The enzyme activity on the antibody bound fraction is inversely proportional to the native antigen concentration. By utilizing several different serum references of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

Procedure:

Prior to assay, the reagents, were allowed to stand at room temperature. The reagents were then gently mixed. The samples prepared according to the "Sample Extraction" procedure.

Formatting of the microplates' wells for each calibrator, control and patient specimen to be assayed in duplicate was carried out. Fifty (50) microlitre (µl) of the appropriate extracted folate standard, control or specimen was added into the assigned wells. Fifty (50) µl of the Folate Enzyme Reagent was added to all the wells. Gently the microplate was mixed for 30 seconds.

Fifty (50) µl of the Folate Biotin Reagent was added to all the wells. The microplate was gently mixed for 30 seconds. It was then covered and incubated for 45 minutes at room temperature.

The contents of the microplate were discarded by decantation. It was then blot dried with absorbent paper. Three hundred and fifty (350) µl of wash buffer was added and decanted each for three (3) times. One hundred (100) µl of substrate reagent was then added to all the wells. It was incubated for 20 minutes at room [temperature. Fifty (50µl) of the stop solution was added to each well. It was gently mixed for 20 seconds. Then the absorbance in each well was read at 450nm.Calculation was done after plotting the graph.

3.8.3 Vitamin B_{12} determination using ELISA kit by Calbiotech.

Principle: Upon mixing the biotinylated antibody with a serum containing the antigen, a reaction results between the antigen and the antibody. After a short incubation, the enzyme conjugate is added which permits an increase in sensitivity for low concentrated samples. Upon the addition of the enzyme conjugate, competition reaction results between the enzyme analog and the antigen in the sample for a limited number of antibody binding sites. A simultaneous reaction between the biotin attached to the antibody and the streptovadin immobilized on the microwell occurs. This effect the separation of the antibody bound fraction after decantation or aspiration. The enzyme activity on the antibody bound fraction is inversely proportional to the native antigen concentration. By utilizing several different serum references of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

Procedure:

Prior to assay, the reagents, were allowed to stand at room temperature. The reagents being gently mixed before use. The samples were then prepared according to the "Sample Extraction" procedure. Formatting of the microplates' wells for each serum reference calibrator, control and patient specimen to be assayed in duplicate was carried out. Fifty (50) µl of the appropriate extracted vitamin B_{12} calibrator, control and specimen was added to the assigned wells. Fifty (50) µl of the vitamin B_{12} Biotin Reagent was added to all the wells. The microplate was gently mixed for 30 seconds. It was then covered and incubated for 45 minutes at room temperature. Fifty (50) µl of vitamin B_{12} Enzyme Reagent was added to all the wells. The microplate was gently mixed for 30 seconds. It was then covered and incubated for 30 minutes at room temperature. The content of the microplate being discarded by decantation. It was then blot dried with absorbent paper. Three hundred and fifty (350) µl of wash buffer was added and decanted each time for three (3) times. One hundred (100) µl of substrate reagent was then added to all the wells. It was incubated for 20 minutes at room temperature. Fifty (50) µl of the stop solution was added to each well and thereafter, was mixed for 20 seconds. Then the absorbance in each well was read at 450nm.Calculation was done after plotting the graph.

3.8.4 Ferritin determination using ELISA kit by Calbiotech.

Principle: This Ferritin ELISA kit is a solid phase sandwich assay method, based on a streptavidin-biotin principle. The standards, samples and the biotinylated Anti-Ferritin Antibody reagent are added into designated wells, coated with streptavidin. Endogenous Ferritin in the patient's serum binds to the antigenic site of the biotinylated Anti-Ferritin Antibody. Simultaneously, the biotinylated antibody is immobilized onto the wells through the high affinity Streptavidin-Biotin interaction. Unbound protein and excess biotin conjugated antibody are washed off by wash buffer. Upon the addition of the Peroxidase (HRP) conjugated Anti-Ferritin Reagent, a sandwich complex is formed, the analyte of interest being in between the two highly specific antibodies, labeled with Biotin and HRP. Unbound protein excess enzyme conjugated antibody reagent is washed off by wash buffer. Upon the addition of the substrate, the intensity of colour developed is directly proportional to the concentration of Ferritin in the samples. A standard curve is prepared relating colour intensity to the concentration of the Ferritin.

Procedure:

Prior to assay, the reagents, were allowed to stand at room temperature. The reagents were gently mixed before use. The desired numbers of coated strips were placed in the holder. And twenty five (25)µl of Ferritin standards, controls and samples were added into appropriate wells.100ul of Biotin Reagent was added into each well. The plate was shaken to mix for 30 seconds. It was then covered and incubated for 30 minutes at room temperature. Liquid was then removed from all the wells. Three hundred (300) µl of wash buffer was used to wash the wells for three (3) times. It was blot dried using an absorbent paper. One hundred (100) µl of Enzyme Reagent was added into each of the well. The microplate was then covered and incubated for 30 minutes at room temperature. Liquid again was removed from all the wells. Three hundred (300) µl of wash buffer was used to wash the wells for three (3) times. It was blot dried using an absorbent paper. And one hundred (100) µl of the substrate was then added to all the wells. It was incubated for 15 minutes at room temperature. Fifty (50) µl of stop solution was then added to all the wells. The microplate being mixed by shaking for 20 seconds. The absorbance was read on ELISA reader at 450nm within 15 minutes after adding the stopping solution.

3.8.5 Serum transferrin receptors determination using ELISA kit by Calbiotech.

Principle: Upon mixing the biotinylated antibody with a serum containing the antigen, a reaction results between the antigen and the antibody. After a short incubation, the enzyme conjugate is added which permits an increase in sensitivity for low concentrated samples. Upon the addition of the enzyme conjugate, competition reaction results between the enzyme analog and the antigen in the sample for a limited number of antibody binding sites. A simultaneous reaction between the biotin attached to the antibody and the streptovadin immobilized on the microwell occurs. This effect the separation of the antibody bound fraction after decantation or aspiration. The enzyme activity on the antibody bound fraction is inversely proportional to the native antigen concentration. By utilizing several different serum references of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

Procedure:

Prior to assay, the reagents, were allowed to stand at room temperature. Thereafter, the reagents were gently mixed before use. Formatting of the microplates' wells for each standard, control and patient specimen to be assayed in duplicate was carried out. Ten (10µl) of appropriate serum reference calibrator, control or specimen was added into the assigned

well. Then, 100ul of the sTfR Biotin Reagent was added to all the well and the microplate swirled gently for 30 seconds to mix. Thereafter, it was covered and incubated for 45 minutes at room temperature.

The content of the micro plate was then discarded by decantation and blot dried with absorbent paper. Three hundred and fifty (350) µl of wash buffer was then added and decanted each time for three (3) times. And one hundred (100) µl of Anti-sTfR Enzyme Reagent was added to all the wells. It was then covered and incubated for 30 minutes at room temperature. The contents of the micro plate again were discarded by decantation and blot dried with absorbent paper. Also, 350µl of wash buffer was added and decanted each time for three (3) times. And one hundred (100) µl of the substrate solution was added to all the wells and incubated for 15 minutes at room temperature. Fifty (50) µl of stop solution was then added to each of the well and the microplate gently mixed for 20 seconds. Absorbance in each well was then read at 450nm.

3.8.6 Total protein estmation using direct biuret method (Wu, 2006)

Principle: At alkaline pH value, proteins form a stable complex with copper II ions, which is photometrically measured.

Procedure:

Into three (3) different tubes, 0.05ml each of the sample, blank and standard was put. And 2.5ml of the reagent was then added to each of the tubes.

It was well mixed and incubated for 20 minutes at room temperature.

The absorbance was then read at a wavelength of 540nm.

Calculation: Optical density of sample/Optical density of standard x5 gives the total protein in gramme/litre.

3.8.7 Albumin estimation using bromocresol green method (Wu, 2006)

Principle: At a pH value, albumin is specifically combined with bromocresol green to produce a coloured complex which is photometrically measured.

Procedure:

Each of the sample, blank and standard reagents was put 0.02ml in three (3) different tubes. Five (5.0) ml of the bromocresol green reagent was then added to each of the tubes. It was

well mixed and incubated for 5 minutes at room temperature. The absorbance was then read immediately at a wavelength of 630nm.

Calculation:

Optical density of sample/Optical density of standard x 5 gives the albumin in gramme/litre.

3.8.8 Serum iron estimation using techo kits.

Principle; The iron in serum is dissociated from its Fe (III) - transferrin complex by the addition of an acidic buffer containing hydroxylamine. This addition reduces the Fe (III) to Fe (II). The chromogenic agent, Ferene, forms a highly colored Fe (II) - complex that is measured photometrically at 560 nm.

Procedure;

Tubes were labeled "Blank", "Standard", "Control", "Sample", etc. To all the tubes, 2.5 ml Iron Buffer reagent was added. Thereafter, 0.5ml (500µl) sample was added to respective tubes and mixed and same volume iron free water was added to blank. Spectrophotometer was zeroed at 560nm with the reagent blank. Absorbances of all tubes were recorded (A1reading). Fifty (50) µl iron colour reagent was added to all the tubes and mixed. All the tubes were placed in the heating bath at 37^{oC} for 10 minutes. The instrument was zeroed at 560nm with the reagent blank.Absorbances of all the tubes were read (A2 reading).

Calculations:

A2 test-A1 test ÷ A2 STD – A1 STD x Conc. of Std = Total Iron (µg/dl)

3.8.9 UIBC (Unsaturated Iron Binding Capacity)

Principle: The unsaturated iron binding capacity (UIBC) is determined by adding Fe (II) iron to serum so that they bind to the unsaturated iron binding sites on transferrin. The excess Fe (II) ions are reacted with Ferrozine to form the color complex, which is measured photometrically. The difference between the amount of Fe (II) added and the amount of Fe (II) measured represents the unsaturated iron binding.

Procedure:

Tubes were labelled blank, standard control and test. Two (2.0) ml UIBC buffer reagent was added to all the tubes. To "Blank" 1.0 ml iron free water was added and mixed. To "Standard" 0.5ml iron free water plus 0.5ml (500µl) Standard was added and mixed. To "Test" 0.5ml (500µl) respective sample plus 500µl of Iron Standard was added and mixed. The spectrophotometer was zero at 540nm with reagent blank. The absorbance of all the tubes were read and recorded as A1 reading. Fifty (50) µl of iron colour reagent was added to

all the tubes and mixed. The tubes were placed in the waterbath at 37^{0C} for 10 minutes. The spectrophotometer was zero at 560nm using the reagent blank. The absorbance of all the tubes were read and recorded as A2 reading.

Calculations:

Concentration of Std – A2 test –A1 test ÷ A2 Std – A1 Std x Concentration of Std = UIBC (µg/dl).

3.8.10 Calculations for total iron binding capacity (TIBC):

The total iron binding capacity (TIBC) is determined by adding the serum iron value to the UIBC value.

Therefore, TIBC (µg/dl) = Iron level + UIBC

3.8.11 Calculations for transferrin saturation (TS):

TS= the ratio of iron to total iron binding capacity (TIBC) expressed in percentage.

3.9 Statistical analysis

Statistical analysis was done using Statistical package for social sciences (SPSS) version 20. The variables were expressed in mean and standard deviation with categorical data as frequencies and percentages. The differences between means of more than two groups were tested by performing analysis of variance (ANOVA). Student's t-test was used for group patterns comparison between two groups only. Pearson correlation coefficients were calculated to determine correlation of serum ferritin, TIBC, UIBC, percent transferrin saturation against serum iron and serum ferritin against total protein, albumin, folate, vitamin B12, body mass index, TIBC, UIBC, percent transferrin saturation and serum iron.

CHAPTER FOUR

RESULTS

4.1 Demographic characteristics of study participants

Table 4.1 shows the demographic characteristics of study participants. A total of 72 pregnant women participated in the study. Those in the 18-29 years age group accounted for 52.78%, followed by the 30-41 age group (47.22%). Majority of them attained tertiary level of education (73.61%), whereas 18 (26.39%) attended up to secondary level of education. Fifty-six (41.67%) were in the parity category of primigravidae while 42 (58.33%) were in the parity category of 2 and above. Most of the study participants 44 (61.11%) had household size of at most 3 while 28 (38.89%) had home size of equal to or greater than 4. The proportion of unemployed study participants was 31(43.06%), 21(29.17%) had average monthly income between 10,000 and 49,999 naira, 7(9.72%) earn between 50,000-9,999 while 13(18.05%) earn >100,000 naira monthly.

Table 4.1: Demographic characteristics of study participants

Variables	Frequency(N=72)	Percentage (%)
Age group/years		
18-29	38	52.78
30 -41	34	47.22
Education		
Secondary	19	26.39
Tertiary	53	73.61
Parity		
Once	30	41.67
2 and above	42	58.33
Household size		
<4	44	61.11
>4	28	38.89
Average monthly income/Naira		
10,000-49,999	21	29.17
50,000-99,999	7	9.72
>100,000	13	18.05
Unemployed	31	43.05

4.2 : Haemoglobin (Hb), packed cell volume (PCV) and red cell indices of the pregnant women across the trimesters and the non pregnant women (control).

The result from this study showed a significant decrease (p = 0.01) in the PCV of the pregnant women in the 1st trimester (33.97 ±3.10)%, 2nd trimester (32.52 ±3.02)% and the third trimester (33.23 ±2.32)% when compared to the control (37.52 ±2.09)%, but no significant change between the different trimesters. Similarly, the result of the haemoglobin concentration showed a significant decrease (p = 0.01) in the1st trimester (11.10 ±1.43) g/dl, 2nd trimester (10.83 ±1.62)g/dl and the 3rd trimester (11.13 ±1.10)g/dl when compared to the control (12.02 ±1.02)g/dl but no significant changes between the different trimesters. Mean cell haemoglobin (MCH) concentration in the 1st, 2nd and 3rd trimesters (29.19 ±3.07)pg, (27.78 ±4.15)pg, (29.13 ±3.28)pg showed no significant changes (p = 0.088, p = 0.887, p = 0.0849) respectively across the trimesters and when compared to the control group (29.60±3.01). Mean cell haemoglobin concentration (MCHC) in the 1st, 2nd and 3rd trimesters (32.53 ±2.79) g/dl, (33.00 ±3.03)g/dl, (33.60 ±2.56)g/dl showed no significant change (p = 0.713, p = 0.189, p = 0.131) respectively, across the trimesters and when compared to the control (32.06 ±2.06)g/dl. Similarly, mean cell volume (MCV) did not show any significant change across the trimesters but showed a significant decrease (p = 0.001) in the 1st and 2nd trimester (86.03 ±7.75fl, 83.72 ±8.81)fl respectively when compared to the control (92.60 ±9.82)fl.

Table 4.2: Comparison of the Hb, PCV and red cell indices of the pregnant women across trimesters and the non pregnant women.

	Hb(g/dl)	PCV(%)	MCV(fl)	MCH(pg)	MCHC(g/dl)
Trimester	**Mean±SD**	**Mean±SD**	**Mean±SD**	**Mean±SD**	**Mean±SD**
1st trimester (n=72)	11.10±1.43	33.97±3.10	86.03±7.75	29.19±3.07	32.53±2.79
2nd trimester (n=69)	10.83±1.62	32.52±3.02	83.72±8.81	27.78±4.15	33.00±3.03
3rd trimester (n=68)	11.13±1.10	33.23±2.32	86.96±9.82	29.13±3.28	33.60±2.56
Non- pregnant (n=50)	12.02±1.02	37.52±2.09	92.67±10.03	29.60±3.01	32.06±2.06
F (p) value	8.22(0.01)	36.61(0.01)	9.63(0.01)	3.40(0.018)	3.65(0.013)
1st vs 2nd P-value	0.718	0.028	0.375	0.107	0.772
1st vs 3rd P-value	0.986	0.376	0.925	0.999	0.088
1st vs NP P-value	0.01	0.01	0.001	0.887	0.713
2nd vs 3rd P-value	0.465	0.481	0.183	0.156	0.595
2nd vs NP P-value	0.01	0.01	0.001	0.887	0.189
3rd vs NP P-value	0.01	0.01	0.015	0.849	0.131

Key: 1st vs 2nd = 1st trimester versus 2nd trimester
1st vs 3rd = 1st trimester versus 3rd trimester
1st vs NP = 1st trimester versus non pregnant
2nd vs 3rd = 2nd trimester versus 3rd trimester
2nd vs NP = 2nd trimester versus non pregnant
3rd vs NP= 3rd trimester versus non pregnant
N = number of pregnant women
NP = non-pregnant women
P<0.01 = significant
Hb = haemoglobin
PCV = packed cell volume
MCV = mean cell volume
MCH = mean cell haemoglobin
MCHC = mean cell haemoglobin concentration

4.3 White blood cell (WBC), platelets, and absolute LYM, MXD and NEUT of the pregnant women across the trimesters and the non pregnant women (control).

The mean absolute lymphocytes (LYM) count of the pregnant women did not show any significant changes across the trimesters though were increased when compared to the control. The 1st, 2nd and in the 3rd trimester (2.0±1.0) x10^3/µl, (2.0 ±0.6) x10^3/µl (2.0±0.5) x10^3/µl respectively showed significant increase (p = 0.01), when compared to the control (0.4 ±0.4) x10^3/µl. The total white blood cell count (WBC) in the 1st, 2nd and 3rd trimesters (8.0 ±2.0) x 10^3/µl), (7.0±2.0) x 10^3/µl, (7.0 ±2.0) x 10^3/µl respectively were increased in comparison with the control (2.0 ±1.0) x 10^3/µ though there was no significant change between the different trimesters. Platelets, on the other hand, decreased significantly (p = 0.01) in the 2nd and 3rd trimesters (208.0 ±56.0) x10^3/µl, and (209.0 ±64.0) x10^3/µl respectively when compared to the 1st trimester and the control (256.0 ±59.0) x10^3/µl. The MXD (monocyte, eosinophil and basophil) showed a significant decrease (p = 0.01) in the 1st, 2nd and 3rd trimesters (2.0±1.3, 1.0 ±1.0, 1.0 ±0.4) x10^3µl respectively, when compared to the control (3.0 ±1.1) x10^3/µl. Neutrophil showed no significant difference (p = 0.899) in the 1st, 2nd and 3rd trimesters (5.0 ±2.0, 5.0±2.0, 5.0±2.0) x10^3/µl when compared to the control (5.0 ±1.3) x 10^3/µl.

Table 4.3: Comparison of the WBC, platelets and absolute lymphocytes, mixed and neutrophils count of the haematological parameters of the pregnant women across the trimesters and the non pregnant women.

	LYM X10^3/µl)	M X D (X10^3/µl)	NEUT (X10^3/µl)	WBC (X10^3//µl)	PLT (X10^3/µl)
Trimester	**Mean ± SD**	**Mean ±SD**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**
1st trimester (n=72)	2.0±1.0	2.0±1.3	5.0±2.0	8.0±2.0	242.0 ±68.0
2nd trimester (n=69)	2.0±0.6	1.0±1.0	5.0±2.0	7.0±2.0	208.0±56.0
3rd trimester (n=68)	2.0±0.5	1.0±0.4	5.0±2.0	7.1±2.2	209.0± 64.0
Non pregnant (n=50)	0.4±0.4	3.0± 1.1	5.0±1.3	2.0± 1.0	256.0±59.0
F(P) value	56.13 (0.01)	58.67(0.01)	0.196 (0.899)	117.88(0.01)	9.75(0.01)
1st vs 2nd P-value	0.128	0.605	0.964	0.937	0.006
1st vs 3rd P-value	0.056	1.00	1.00	0.952	0.015
1st vs NP P-value	0.01	0.01	0.968	0.01	0.610
2nd vs 3rd P-value	0.952	0.256	0.951	0.996	1.00
2nd vs NP P-value	0.01	0.01	0.992	0.01	0.01
3rd vs NP P-value	0.01	0.01	0.991	0.01	0.01

Key: 1st vs 2nd = 1st trimester versus 2nd trimester
1st vs 3rd = 1st trimester versus 3rd trimester
1st vs NP = 1st trimester versus non pregnant
2nd vs 3rd = 2nd trimester versus 3rd trimester
2nd vs NP = 2nd trimester versus non pregnant
3rd vs NP = 3rd trimester versus non pregnant
N = Number of pregnant women
NP = Non-pregnant women
LYM = lymphocytes
MXD = mixed
NEUT = neutrophils
P<0.01 = significant

4.4 Some nutritional indices across the trimesters among pregnant women in comparison with the non pregnant women (control).

The mean total protein of the test group decreased as pregnancy progressed from the 1st, 2nd and 3rd trimesters (74.42 ±12.36) g/dl, (72.30 ±44.17) g/dl and (60.51 ±4.07) g/dl respectively. This depicts that there was a decrease in total protein value with increase in gestational age though the mean difference between the 1st (74.42 ±12.36) g/dl and 2nd trimester (72.30 ±44.17) g/dl was not statistically significant (p = 0.981). Similarly, the mean total protein of the pregnant women in the 1st trimester (74.42 ±12.36) g/dl and the non pregnant control (75.16±4.81) g/d showed no statistical significance (p =0.967). There was a significant decrease in the 3rd trimester (60.51 ±4.07) g/dl when compared to the values in the 1st and 2nd trimesters (74.42 ±12.36) g/dl, (72.30 ±44.17) g/dl and the control (75.16±4.81) g/dl respectively. The mean albumin level showed also a decrease as pregnancy progressed from the 1st to 2nd and 3rd trimesters: (39.56 ±7.72) g/dl, (28.81 ±10.39)g/dl, (24.32 ±3.12) g/dl respectively and the control group (43.48 ±2.71)g/dl. There was a statistically significant decrease from the 1st and 2nd trimester, 1st and 3rd trimester of pregnancy (p=0.01). Folate concentration decreased in the 1st and 2nd trimesters when compared to the control and the fall was markedly significant in the 2nd trimester. There was no significant difference (p=0.869) in the 1st (18.94 ±10.35)ng/ml when compared to the 3rd trimester (20.06+9.50) ng/ml but showed a significant decrease (p = 0.001) in the 2nd trimester (14.82+8.19) ng/ml when compared to the 3rd trimester (20.06+9.50) ng/ml and the control (20.13 ±7.82) ng/ml. The serum vitamin B_{12} concentration of the study participants in the 2nd trimester (666.62 ±258.93) pg/ml was lower than the values in the 1st and 3rd trimesters (788.14 ±317.44)pg/ml and (699.42 ±506.69) pg/ml respectively though not statistically significant. But the value of the 2nd (666.62 ±258.93) pg/ml was significantly lower (p = 0.001) than that of the non pregnant control (871.90 ±279.46) pg/ml. Similarly, the body mass index (BMI) of the study participants showed no significant difference across the trimesters but showed a significant difference in the 2nd trimesters (29.49 ±5.05) kg/m^2 when compared to the control (28.33 ±4.91) kg/m^2 and also a significant increase in the 3rd trimester (30.54 ±5.15) kg/m^2 when compared to the control (28.33 ±4.91) kg/m^2.

Table 4.4: Some nutritional indices across the trimesters of pregnant women in comparison with non pregnant women.

	Total protein (g/l)	Albumin (g/l)	Folate (ng/ml)	Vit B_{12}(pg/ml)	BMI (kg/m^2)
Trimester	**Mean ± SD**	**Mean ± SD**	**Mean±SD**	**Mean±SD**	**Mean ± SD**
1st trimester (n=72)	74.42 ± 12.36	39.56±7.72	18.94±10.35	788.14±317.44	28.35±4.93
2nd trimester (n=69)	72.30 ± 44.17	28.81±10.39	14..82±8.19	666.62±258.93	29.49±5.05
3rd trimester (n=68)	60.51 ± 4.07	24.32±2.71	20.06 ±7.82	699.42 ±506.69	30.54±5.15
Non pregnant (n=50)	75.16 ± 4.81	43.48 ± 2.71	20.13 ± 9.50	871.90 ±279.46	28.33±4.91
F(P) value	5.34 (0.01)	100.42(0.01)	5.11(0.002)	3.89(0.01)	9.59(0.01)
1st vs 2nd P-value	0.981	0.01	0.047	0.065	0.569
1st vs 3rd P-value	0.01	0.01	0.869	0.607	0.053
1st vs NP P-value	0.967	0.001	0.927	0.418	0.016
2nd vs 3rd P-value	0.131	0.005	0.001	0.964	0.582
2nd vs NP P-value	0.951	0.01	0.012	0.001	0.01
3rd vs NP P-value	0.01	0.01	1.00	0.091	0.01

Key: 1st vs 2nd = 1st trimester versus 2nd trimester
1st vs 3rd = 1st trimester versus 3rd trimester
1st vs NP = 1st trimester versus non pregnant
2nd vs 3rd = 2nd trimester versus 3rd trimester
2nd vs NP = 2nd trimester versus non pregnant
3rd vs NP = 3rd trimester versus non pregnant
N = Number of pregnant women
NP = Non-pregnant wome,
P<0.01 = significant

4.5 Iron levels of the pregnant women across the trimesters in comparison with the control

The serum ferritin level of the pregnant women decreased across the trimesters. The mean serum ferritin levels in the 2nd and 3rd trimesters (34.23 ±41.87) ηg/ml, (30.26 ±20.41) ηg/ml respectively, showed significant decrease (p=0.012, p=0.01) when compared to the 1st trimester (65.74 ±54.83) ηg/ml. However, the fall in serum ferritin level was markedly significant (p=0.01) in the 3rd trimester (30.26±20.41) ηg/ml when compared to the control (60.46+47.00) ηg/ml. Similarly, the serum iron level of the test group showed a significant decrease (p = 0.01) in the 2nd trimester (70.30 ±29.98) µg/dl when compared to the 1st trimester (89.94±23.03) µg/dl. There was no significant difference (p = 0.092, p = 0.078) in the 1st (89.94±23.03) µg/dl and 2nd (70.30±29.98) µg/dl trimesters respectively, when compared to the nonpregnant women (81.10 ±18.38) µg/dl. There was a significant increase (p = 0.01) in the serum iron level in the 3rd trimester (121.50 ±20.74) µg/dl when compared to the 1st (89.94±23.03) µg/dl, 2nd trimester (70.30 ±29.98) µg/dl and the control (81.10 ±18.38) µg/dl. Conversely, the values for serum transferrin receptors (sTfR) in the 1st, 2nd and 3rd trimesters (23.86 ±7.57) nmol/ml, (22.87 ±5.47) nmol/ml, (23.60 ±6.86) nmol/ml respectively showed no significant difference (p = 0.416) among the pregnant women and the control. Similarly, total iron binding capacity (TIBC) across the trimesters (507.86 ±39.27) µg/dl, (515.38 ±42.52) µg/dl, (499.33 ±53.84) µg/dl showed no significant difference (p = 0.019) among the pregnant women and the non pregnant women (506.54±30.68) µg/dl. The unsaturated iron binding capacity (UIBC) was observed to show a significant increase (p = 0.01) in the 2nd trimester (446.22 ±44.11) µg/dl when compared to the 1st trimester (418.93 ±38.39) µg/dl. There was a significant decrease (p = 0.01) in the 3rd trimester (377.75 ±47.56) µg/dl when compared to the control (425.02 ±24.23) µg/dl but showed no significant difference (p= 0.707, p=0.006) respectively between the values in the 1st and 2nd trimesters when compared to the control. Similarly, there was a significant decrease (p=0.01) in the percent transferrin saturation of the test group in the 2nd trimester (13.59+5.69)% when compared to the 1st trimester (17.42±4.33)%, and 3rd trimester (24.37±3.69)%. There was also a significant increase in the percent transferrin saturation (TS%) in the 3rd trimester (24.37±3.69)% when compared to the control (15.80±3.14)% but there was no significant difference in the mean values of the 1st and 2nd trimesters (p=0.085, p= 0.039) respectively when compared to the non pregnant women (15.80±3.14)%.

Table 4.5: Iron levels of pregnant women across the trimesters in comparison with the non pregnant women.

	Ferritin (ng/ml)	Serum iron (µl /dl)	sTfR (nmol/ml)	UIBC (µl/dl)	TIBC (µl/dl)	TS(%)
Trimester	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**
1st trimester (n=72)	65.74± 54.83	89.94±23.03	23.86±7.57	418.93±38.39	507.86± 39.27	17.42±4.33
2nd trimester (n=69)	34.23±41.87	70.30±29.98	22.87±5.47	446.22±44.11	515.38±42.52	13.59±5.6
3rd trimester (n=68)	30.26±20.41	121.50±20.74	23.60±6.86	377.75±47.56	499.33±53.84	24.37±3.69
Non pregnant (n=50)	60.46±47.00	81.10±18.38	25.08±8.78	425.02±24.23	506.54±30.68	15.80±3.14
F(P) value	11.71(0.01)	57.66(0.01)	0.953(0.416)	34.04(0.01)	1.59(0.19)	75.22(0.01)
1st vs 2nd P-value	0.001	0.01	0.808	0.01	0.697	0.01
1st vs 3rd P-value	0.01	0.01	0.997	0.01	0.711	0.01
1st vs NP P-value	0.941	0.092	0.850	0.707	0.997	0.085
2nd vs 3rd P-value	0.894	0.01	0.900	0.01	0.219	0.01
2nd vs NP P-value	0.012	0.078	0.384	0.006	0.554	0.039
3rd vs NP P-value	0.01	0.01	0.747	0.01	0.795	0.01

Key:1st vs 2nd = 1st trimester versus 2nd trimester
1st vs 3rd = 1st trimester versus 3rd trimester
1st vs NP = 1st trimester versus non pregnant
2nd vs 3rd = 2nd trimester versus 3rd trimester
2nd vs NP = 2nd trimester versus non pregnant
3rd vs NP = 3rd trimester versus non pregnant
N = Number of pregnant women
NP = Non-pregnant women
P<0.01 = significant

4.6 Relationship between the demographic characteristics with the haematological and nutritional parameters measured.

Table 4.6 represents the haematological and nutritional indices among pregnant women in respect of age in the 1st trimester. Between the age groups 18-29 years and 30 and above years, there was no significant difference ($p>0.05$).

Tables 4.7 represent the haematological and nutritional parameters among the pregnant women in respect of educational level attained in the 1st trimester. There was no significant difference between the two groups (secondary and tertiary).

The haematological and nutritional parameters among the pregnant women in respect of parity. Table 4.8 showed that there was significant difference in the Hb, platelets, and BMI ($p=0.014$, $p=0.023$, $p=0.047$) in respect of parity among the two groups (once and more than once) in the 1st trimester.

Table 4.6: Haematological parameters among the pregnant women in respect of age in the 1st trimester.

	Age (years)		
	18-29 (n-38)	30-41 (n=34)	
Parameters	**Mean ± SD**	**Mean ± SD**	**p-value**
Hb(g/dl)	11.18±0.63	11.00±1.18	0.189
PCV (%)	33.97±3.51	34.09±2.55	0.330
MCV (fl)	84.24±6.95	88.02±8.19	0.098
MCH(pg)	28.94± 2.99	29.47±3.20	0.428
MCHC(g/dl)	32.86 ±2.67	32.14±2.91	0.628
LYM($X10^3/\mu l$)	1.90 ±1.02	1.66±0.88	0.915
MXD ($X10^3/\mu l$)	1.85±1.42	2.03±1.17	0.310
NEUT($X10^3/\mu$)	5.08 ±1.56	4.53±1.31	0.344
WBC($X10^3/\mu l$)	8.18±1.99	7.73±1.84	0.541
PLT ($X10^3/\mu l$)	240.74±66.77	242.27±67.29	0.763
Total protein (g/dl)	72.62±11.05	76.55±13.61	0.468
Albumin (g/l)	38.87± 7.93	40.36±7.51	0.584
Folate (ng/ml)	18.72±11.10	19.21±9.56	0.128
Vit B_{12} (pg/ml)	711.10±282.12	879.18±336.57	0.567
BMI (kg/m^2)	27.18±4.35	29.73±5.28	0.400
Ferritin (ng/ml)	57.15 ±46.42	75.88±62.58	0.288
Serum iron ($\mu g/dl$)	91.28 ± 20.77	88.36 ± 25.68	0.701
sTfR($nmol/ml$)	23.23 + 8.25	24.61 ± 6.73	0.179
UIBC ($\mu g/dl$)	421.85 ± 41.69	415.48 ± 34.39	0.322
TIBC ($\mu g/dl$)	511.36 ± 42.83	503.73 ± 34.79	0.248
TS (%)	17.62 ± 4.02	17.18 ± 4.73	0.536

WBC = White blood cell count, HB = haemoglobin, PCV = haematocrit, PLT = platelet, LYM = absolute lymphocyte, MXD = absolute mixed count, and NEUT = absolute neutrophil count, MCV = mean cell volume, MCH = mean cell haemoglobin, and MCHC = mean cell haemoglobin concentration.UIBC = unsaturated iron binding capacity, sTfR = serum transferrin receptor, Vit B_{12} = vitamin B12, TIBC = total iron binding capacity, TS = transferrin saturation, BMI = body mass index.

Table 4.7 Haematological and nutritional parameters among the pregnant women in respect of educational level attained in the 1st trimester.

	Secondary (n-19)	Tertiary (n=53)	
Parameters	**Mean ± SD**	**Mean ± SD**	**p-value**
Hb(g/dl)	10.95 ± 1.39	11.15 ± 1.45	0.934
PCV (%)	33.42 ± 2.99	34.17 ± 3.14	0.806
MCV (fl)	84.79 ± 7.12	86.47 ± 7.98	0.272
MCH(pg)	29.68 ± 3.20	29.02 ± 3.05	0.890
MCHC(g/dl)	32.37 ± 3.04	32.58 ± 2.73	0.888
LYM($X10^3/\mu l$)	1.63 ± 1.34	2.04 ± 1.29	0.441
MXD ($X10^3/\mu l$)	1.73 ± 0.65	1.81 ± 1.06	0.052
NEUT($X10^3/\mu l$)	4.68 ± 1.38	4.89 ± 1.51	0.875
WBC($X10^3/\mu l$)	8.16 ± 1.80	7.91 ± 1.97	0.672
PLT ($X10^3/\mu l$)	251.95 ± 82.28	238.15 ± 59.80	0.206
Total protein (g/dl)	77.16 ± 17.27	73.43 ± 10.07	0.096
Albumin (g/l)	40.26 ± 8.47	39.30 ± 7.39	0.545
Ferritin (ng/ml)	84.53 ± 55.32	59.00 ± 53.57	0.536
sTfR($nmol/ml$)	24.68 ± 7.27	23.56 ± 7.72	0.863
Folate (ng/ml)	20.84 ± 11.01	18.26 ± 10.13	0.313
Vit B_{12} (pg/ml)	825.63 ± 35.54	774.70 ± 306.77	0.611
Serum iron ($\mu g/dl$)	87.32 ± 29.44	90.89 ± 20.50	0.261
UIBC ($\mu g/dl$)	409.21 ± 41.18	422.42 ± 37.12	0.674
TIBC ($\mu g/dl$)	495.53 ± 43.29	512.28 ± 3.99	0.197
TS (%)	17.21 ± 5.27	17.49 ± 3.99	0.197
BMI (kg/m^2)	24.47 ± 5.23	27.94 ±4.80	0.840

WBC = White blood cell count, HB = haemoglobin, PCV = haematocrit, PLT = platelet, LYM = absolute lymphocyte, MXD = absolute mixed count, and NEUT = absolute neutrophil count, MCV = mean cell volume, MCH = mean cell haemoglobin, and MCHC = mean cell haemoglobin concentration.UIBC = unsaturated iron binding capacity, sTfR = serum transferrin receptor, Vit B_{12} = vitamin B12, TIBC = total iron binding capacity, TS = transferrin saturation, BMI = body mass index.

Table 4.8 The haematological and nutritional parameters among the pregnant women in respect of parity of the study participants in the 1st trimester.

	Once (n-30)	More than one (n=42)	
Parameters	**Mean ± SD**	**Mean ± SD**	**p-value**
Hb(g/dl)	11.37 ± 1.77	10.94 ± 1.10	0.014
PCV (%)	34.40 ± 3.68	33.67 ± 2.61	0.143
MCV (fl)	84.93 ± 7.21	86.81 ± 8.11	0.602
MCH(pg)	28.53 ± 3.25	29.67 ± 2.89	0.456
MCHC(g/dl)	32.77 ± 2.80	32.36 ±2.80	0.462
LYM($X10^3/\mu l$)	2.10 ± 1.35	1.81 ±1.27	0.736
MXD ($X10^3/\mu l$)	1.99 ± 1.12	1.67 ± 0.82	0.181
NEUT($X10^3/\mu l$)	5.10 ± 1.35	4.64 ± 1.43	0.853
WBC($X10^3/\mu l$)	8.23 ± 1.86	7.79 ± 1.96	0.365
PLT ($X10^3/\mu l$)	236.87 ± 43.96	245.31 ± 78.58	0.023
Total protein (g/dl)	74.73 ± 11.66	74.19 ± 12.97	0.889
Albumin (g/l)	40.70 ± 6.61	38.74 ± 8.41	0.082
Ferritin (ng/ml)	60.23 ± 39.22	69.67 ± 63.88	0.037
sTfR($nmol/ml$)	22.17 ± 6.93	25.07 ± 7.85	0.637
Folate (ng/ml)	20.73 ± 10.60	17.67 ±10.10	0.454
Vit B_{12} (pg/ml)	846.50±353.48	746.45± 286.09	0.213
Serum iron ($\mu g/dl$)	86.93 ± 22.81	92.10 ± 23.21	0.854
UIBC ($\mu g/dl$)	424.93 ± 34.13	414.64 ± 41.02	0.202
TIBC ($\mu g/dl$)	509.33 ± 40.00	506.81 ± 39.19	0.923
TS (%)	16.70 ± 4.08	17.93 ±4.48	0.581
BMI (kg/m^2)	27.33 ± 3.45	29.07 ± 5.69	0.047

WBC = White blood cell count, HB = haemoglobin, PCV = haematocrit, PLT = platelet, LYM = absolute lymphocyte, MXD = absolute mixed count, and NEUT = absolute neutrophil count, MCV = mean cell volume, MCH = mean cell haemoglobin, and MCHC = mean cell haemoglobin concentration.UIBC = unsaturated iron binding capacity, sTfR = serum transferrin receptor, Vit B_{12} = vitamin B12, TIBC = total iron binding capacity, TS = transferrin saturation, BMI = body mass index.

Table 4.9 represents the distribution of the haematological and nutritional parameters among the pregnant women in respect of employed and unemployed study participants in the 1st trimester. There was no significant difference ($p > 0.05$) in the parameters measured.

Conversely, there was significant difference in the serum iron ($p=0.003$) and UIBC levels ($p=0.002$) of the pregnant women in respect of the age groups in the 2nd trimester (table 4.10).

Similarly, 4.11 represents the distribution of the haematological and nutritional parameters among the pregnant women in respect of the educational level attained in the 2nd trimester. There was a significant difference in the MCHC ($p = 0.05$) and albumin ($p = 0.017$) levels of the two groups (secondary and tertiary). Interestingly, 4.18 did not show any siginificant difference ($p>0.05$) in the haematological parameters between the employed and the unemployed.

Table 4.9 Haematological and nutritional parameters among the pregnant women in respect of employed and unemployed in the 1st trimester.

	Employed (n-41)	Unemployed (n=31)	
Parameters	**Mean ± SD**	**Mean ± SD**	**p-value**
Hb(g/dl)	11.15 ± 1.35	11.03 ± 1.54	0.885
PCV (%)	33.90 ± 2.88	34.06 ± 3.42	0.825
MCV (fl)	88.51 ± 7.12	85.39 ± 8.59	0.878
MCH(pg)	29.46 ± 2.94	28. 84 ± 3.28	0.102
MCHC(g/dl)	32.80 ± 2.94	32.16 ± 2.58	0.389
LYM($X10^3/\mu l$)	1.80 ± 0.93	1.77 ± 1.02	0.853
MXD ($X10^3/\mu l$)	2.00 ± 1.38	1.84 ± 1.21	0.842
NEUT($X10^3/\mu l$)	4.83 ± 1.50	4.84 ± 1.46	0.850
WBC($X10^3/\mu l$)	7.90 ± 1.80	8.06 ± 2.10	0.185
PLT ($X10^3/\mu l$)	239.76 ± 68.48	244.48 ± 63.90	0.259
Total protein (g/dl)	75.56 ± 12.74	72.90 ± 11.87	0.880
Albumin (g/l)	38.76 ± 7.58	40.61 ± 7.91	0.959
Ferritin (ng/ml)	64.44 ± 59.07	67.45 ±49.58	0.600
sTfR($nmol/ml$)	24.49 ±7.14	23.03 ± 8.16	0.378
Folate (ng/ml)	17.61 ± 9.90	20.71 ±10.84	0.307
Vit B_{12} (pg/ml)	789.34 ± 343.11	786.55 ± 285.50	0.845
Serum iron ($\mu g/dl$)	92.29 ±24.07	86.84 ± 21.56	0.205
UIBC ($\mu g/dl$)	420.12 ± 40.84	417.35 ± 35.48	0.231
TIBC ($\mu g/dl$)	511.46 ± 42.52	503.10 ± 34.61	0.231
TS (%)	17.73 ± 4.36	17.00 ± 4.33	0.824
BMI (kg/m^2)	29.41 ± 4.70	26.94 ± 4.95	0.738

WBC = White blood cell count, HB = haemoglobin, PCV = haematocrit, PLT = platelet, LYM = absolute lymphocyte, MXD = absolute mixed count, and NEUT = absolute neutrophil count, MCV = mean cell volume, MCH = mean cell haemoglobin, and MCHC = mean cell haemoglobin concentrationUIBC = unsaturated iron binding capacity, sTfR = serum transferrin receptor, Vit B_{12} = vitamin B_{12}, TIBC = total iron binding capacity, TS = transferrin saturation, BMI = body mass index.

Table 4.:10 Haematological and nutritional parameters among the pregnant women in respect of age in the 2nd trimester

	Age (years)		
	18-29 (n=38)	**30-41 (n=31)**	
Parameters	**Mean±SD**	**Mean±SD**	**p-value**
Hb(g/dl)	10.71±1.59	10.97±1.66	0.880
PCV (%)	32.53±3.15	32.52±2.01	0.525
MCV (fl)	83.68±900	83.77±8.73	0.794
MCH(pg)	27.53±4.o7	28.10±4.29	0.745
MCHC(g/dl)	32.71±2.88	33.35±3.21	0.785
LYM($X10^3/\square l$)	7.16±1.98	7.39±1.86	0.785
MXD ($X10^3/\square l$)	0.74±0.55	0.74±0.46	0.881
NEUT($X10^3/\square l$)	4.74±1.62	4.68±1.60	0.568
PLT ($X10^3/\square l$)	206.79±58.44	208.61±53.41	0.395
Total protein (g/dl)	81.82 ± 52.67	60.65 ± 27.31	0.251
Albumin (g/l)	30.08 ± 10.14	27.26 ± 10. 45	0.551
Ferritin (ng/ml)	35.26± 41.44	32.97 ± 43.04	0.853
sTfR($nmol/ml$)	22.55 ± 5.32	23.26± 5.72	0.805
Folate (ng/ml)	13.95 ±7.65	15.90 ± 8.81	0.379
Vit B_{12} (pg/ml)	664.32 ± 252.42	669.45 ± 270.87	0.117
Serum iron ($\square g/dl$)	66.61 ± 27.01	74.84 ± 33.15	0.003
UIBC ($\square g/dl$)	456.37 ± 38.22	433.77 ± 48.15	0.002
TIBC ($\square g/dl$)	523.13 ± 29.88	505.87 ± 53.18	0.276
TS (%)	12.76 ±5.39	14.16 ± 5.97	0.445
BMI (kg/m^2)	28.21 ± 4.59	30.94 ± 5.27	0.395

WBC = White blood cell count, HB = haemoglobin, PCV = haematocrit, PLT = platelet, LYM = absolute lymphocyte, MXD = absolute mixed count, and NEUT = absolute neutrophil count, MCV = mean cell volume, MCH = mean cell haemoglobin, and MCHC = mean cell haemoglobin concentration.UIBC = unsaturated iron binding capacity, sTfR = serum transferrin receptor, Vit B_{12} = vitamin B_{12}, TIBC = total iron binding capacity, TS = transferrin saturation, BMI = body mass index.

Table 4:11: Haematological and nutritional parameters among the pregnant women in respect of educational level attained in the 2nd trimester.

	Educational Level		
	Secondary (n=18)	**Tertiary (n = 51)**	
Parameters	**Mean±SD**	**Mean±SD**	**p-value**
Hb(g/dl)	11.22 ± 1.73	10.69 ± 1.57	0.501
PCV (%)	33.00 ± 2.95	32.35 ± 3.06	0.998
MCV (fl)	83.39 ±8.87	83.84 ± 8.88	0.801
MCH(pg)	28.50 ± 4.71	27.53 ± 3.95	0.418
MCHC(g/dl)	33.83 ± 3.40	32.71 ±2.67	0.050
WBC (x10^3/μl)	7.67 ± 1.75	7.12 ±1.97	0.674
LYM(X10^3/μl)	2.17 ± 0.71	1.88 ± 0.52	0.238
MXD (X10^3/μl)	0.56 ± 0.51	0.80 ± 0.49	0.120
NEUT(X10^3/μl)	5.06 ± 1.47	4.59 ±1.63	0.654
PLT (X10^3/μl)	211.00 ± 57.23	206.41 ± 55.83	0.783
Total protein (g/dl)	75.44 ± 67.42	71.20 ± 33.21	0.122
Albumin (g/l)	32.72 ±13.56	27.43 ± 8.76	0.017
Ferritin (ng/ml)	39.50 ± 53.90	32.37 ±37.19	0.216
sTfR($nmol/ml$)	22.11 ± 5.93	23.14 ± 5.34	0.535
Folate (ng/ml)	17.17 ± 9.71	14.00 ± 7.52	0.171
Vit B_{12} (pg/ml)	701.28 ±310.96	654.39 ± 240.25	0.484
Serum iron ($\mu g/dl$)	69.56 ± 31.75	70.57 ±29.65	0.531
UIBC ($\mu g/dl$)	438.56 ± 44.28	448.92 ± 44.17	0.819
TIBC ($\mu g/dl$)	508.72 ± 47.05	517.73 ± 41.05	0.364
TS (%)	13.50 ± 5.66	13.63 ± 5.76	0.872
BMI (kg/m^2)	31.11 ± 5.23	28.84 ± 4.91	0.764

WBC = White blood cell count, Hb = haemoglobin, PCV = haematocrit, PLT = platelet, LYM = absolute lymphocyte, MXD = absolute mixed count, and NEUT = absolute neutrophil count, MCV = mean cell volume, MCH = mean cell haemoglobin, and MCHC = mean cell haemoglobin concentrationUIBC = unsaturated iron binding capacity, sTfR = serum transferrin receptor, Vit B_{12} = vitamin B_{12}, TIBC = total iron binding capacity, TS = transferrin saturation, BMI = body mass index.

However, table 4.12 showed a significant difference in the albumin (p= 0.041) and UIBC levels (p= 0.040) among the two groups (employed and the unemployed) of the participants in the 2nd trimester.

Similarly, the distribution of the haematological and nutritional parameters among the two groups of the pregnant women in respect of parity in the 2nd trimester recorded significant difference in the concentrations of PCV (p=0.037), MXD (p=0.025), folate (p = 0.010), vitamin B12 (p=0.011), UIBC (p=0.049) and BMI (p=0.046) (table 4.13).

Table 4.12: Haematological and nutritional parameters among the pregnant women in respect of employed and unemployed in the 2nd trimester.

	Employed (n=38)	Unemployed (n = 31)	
Parameters	**Mean±SD**	**Mean±SD**	**p-value**
Hb(g/dl)	11.00 ± 1.66	10.61 ±1.56	0.951
PCV (%)	32.76 ± 3.23	32.23 ± 2.77	0.753
MCV (fl)	84.87 ± 8.45	82.32 ± 9.19	0.667
MCH(pg)	28.16 ±4.12	27.32 ± 4.21	0.854
MCHC(g/dl)	33.11 ±3.07	32.87 ±3.03	0.884
WBC (x10^3/l)	7.26 ± 2.00	7.26 ± 1.84	0.430
LYM(X10^3/l)	2.05 ± 0.57	1.84 ± 0.58	0.220
MXD (X10^3/l)	2.05 ± 0.57	0.77 ± 0.56	0.547
NEUT(X10^3/l)	4.66 ± 1.71	4.77 ± 1.48	0.193
PLT (X10^3/l)	205.63 ± 50.72	210. 03 ± 62.26	0.261
Total protein (g/dl)	64.16 ± 25.86	82.29 ± 58.40	0.072
Albumin (g/l)	28.08 ± 8.47	29.71 ±12.43	0.041
Ferritin (ng/ml)	31.05 ±38.22	38.13 ±46.29	0.135
sTfR($nmol/ml$)	22.97 ±5.24	22.74 ± 5.84	0.204
Folate (ng/ml)	15.63 ± 7.86	13.84 ± 8.60	0.995
Vit B_{12} (pg/ml)	670.26 ±265.17	662.16 ±255.37	0.877
Serum iron (g/dl)	69.89 ± 30.21	70.81 ± 30.19	0.629
UIBC (g/dl)	454.66 ±37.95	435.87 ± 49.33	0.040
TIBC (g/dl)	522.18 ±40.28	507.03 ± 44.35	0.302
TS (%)	13.24 ± 15.44	14.05 ± 6.04	0.395
BMI (kg/m^2)	30.58 ± 4.73	28.03 ± 5.15	0.642

WBC = White blood cell count, Hb = haemoglobin, PCV = haematocrit, PLT = platelet, LYM = absolute lymphocyte, MXD = absolute mixed count, and NEUT = absolute neutrophil count, MCV = mean cell volume, MCH = mean cell haemoglobin, and MCHC = mean cell haemoglobin concentration.UIBC = unsaturated iron binding capacity, sTfR = serum transferrin receptor, Vit B_{12} = vitamin B_{12}, TIBC = total iron binding capacity, TS = transferrin saturation, BMI = body mass index.

Table 4:13: H**aematological and nutritional parameters among the pregnant women in respect of parity in the 2nd trimester**

	Once (n=30)	More than one (n=39)	
Parameters	**Mean±SD**	**Mean±SD**	**p-value**
Hb(g/dl)	10.77 ± 1.31	10.87 ± 1.84	0.061
PCV (%)	32.50 ± 2.25	32.54 ±3.53	0.037
MCV (fl)	84.30 ± 9.24	83.28 ± 8.57	0.876
MCH(pg)	27.67 ± 3.59	27.87 ± 4.58	0.109
MCHC(g/dl)	32.87 2.75	33.10 ± 3.26	0.332
WBC (x10^3/l)	7.37 ± 2.34	7.18 ± 1.54	0.110
LYM(X10^3/l)	1.93 ± 0.58	1.97 ± 0.58	0.527
MXD (X10^3/l)	0.83 ± 0.46	0.67 ±0.53	0.025
NEUT(X10^3/l)	4.77 ± 1.89	4.67 ±1.36	0.465
PLT (X10^3/l)	213.13 ± 60.46	203.36 ± 52.36	0.146
Total protein (g/dl)	74.90 ± 36.31	70.31 ± 49.76	0.728
Albumin (g/l)	26.00 ± 8.69	30.97 ±11.15	0.316
Ferritin (ng/ml)	33.52 ±41.72	34.77 ± 42.52	0.494
sTfR($nmol/ml$)	23.33 ± 6.00	22.51 ± 5.08	0.081
Folate (ng/ml)	16.00 ±9.55	13.92 ±6.94	0.010
Vit B_{12} (pg/ml)	682.57 ±339.01	654.36 ±178 .64	0.011
Serum iron (g/dl)	69.77 ± 26.78	70.72 ±32.56	0.153
UIBC (g/dl)	454.97 ±38.06	439.49 ± 47.64	0.049
TIBC (g/dl)	521.73 ±32.38	510.49 ± 48.77	0.067
TS (%)	13.40 ±5.19	13.74 ± 6.11	0.261
BMI (kg/m^2)	28.43 ± 3.61	30.21 ±5.86	0.046

WBC = White blood cell count, Hb = haemoglobin, PCV = haematocrit, PLT = platelet, LYM = absolute lymphocyte, MXD = absolute mixed count, and NEUT = absolute neutrophil count, MCV = mean cell volume, MCH = mean cell haemoglobin, and MCHC = mean cell haemoglobin concentration. UIBC = unsaturated iron binding capacity, sTfR = serum transferrin receptor, Vit B_{12} = vitamin B_{12}, TIBC = total iron binding capacity, TS = transferrin saturation, BMI = body mass index.

Third trimester recorded significant difference in the distribution of the haematological and nutritional parameters among the two groups of the pregnant women in respect of age, educational level attained, parity, and among the employed and unemployed participants (tables 4.14-4.17).

MCHC, Hb and sTfR showed significant difference (p=0.022), (p = 0.002) (p-0.015) respectively in respect of age groups of the participants in the 3rd trimester (table 4.14)

Statistically significant difference was noted for MCHC (p=0.006) based on the educational level attained among the study participants (table 4.15). Similarly, table 4.16 depicts that there was significant difference in MCV, MCHC, LYM and BMI (P=0.002), (P=0.016), (p=0.026) and (p=0.045) respectively among the two groups of the pregnant women in respect of parity.

Table 4.17 also showed statistically significant difference in MXD, vitamin B_{12} and percent transferrin saturation (p=0.004), (p=0.048), (p=0.003) respectively among the employed and unemployed groups of the pregnant women in the 3rd trimester.

Table 4.14: Haematological and nutritional parameters among the pregnant women in respect of age in the 3rd trimester.

	Age (years)		
	18-29 (n=37)	30-41 (n =31)	
Parameters	**Mean±SD**	**Mean±SD**	**p-value**
Hb(g/dl)	11.41 ± 1.24	10.89 ±0.85	0.002
PCV (%)	33.68 ± 2.51	32.70 ±1.99	0.284
MCV (fl)	86.32 ± 10.09	87.72 ±9.61	0.963
MCH(pg)	29.30 ±3.53	28.92 ±3.00	0.280
MCHC(g/dl)	34.00 ± 2.83	33.12 ±2.51	0.022
WBC (x10^3/μl)	7.41 ± 2.29	6.68 ± 2.10	0.690
LYM(X10^3/μl)	1.84 ± 0.50	1.71 ± 0.59	0.073
MXD (X10^3/μl)	0.74 ±0.39	0.94 ± 0.44	0.089
NEUT(X10^3/μl)	5.16 ± 2.06	4.39 ± 1.78	0.728
PLT (X10^3/μl)	201. 97 ± 44.97	216.58 ± 80.33	0.186
Total protein (g/dl)	59.89 ± 3.73	61.26 ± 4.39	0.213
Albumin (g/l)	23.84 ± 2.66	24.90 ± 3.54	0.545
Ferritin (ng/ml)	31.92 ±2.66	28.29 ±17.51	0.103
sTfR($nmol/ml$)	25.54 ± 7.44	21.29 ±5.35	0.015
Folate (ng/ml)	20.73 ± 8.41	19.42 ± 7.14	0.169
Vit B_{12} (pg/ml)	555.11 ± 288.38	871.68 ± 645.94	0.069
Serum iron ($\mu g/dl$)	120.70 ± 18.97	122.45 ±22.96	0.195
UIBC ($\mu g/dl$)	380.30 ± 50.29	374.71 ± 44.69	0.272
TIBC ($\mu g/dl$)	501.00 ±53.82	497.35 ± 54.67	0.840
TS (%)	24.14 ± 3.77	24.65 ±3.62	0.730
BMI (kg/m^2)	29.24 ± 4.66	32.10 ± 5.35	0.427

WBC = White blood cell count, Hb = haemoglobin, PCV = haematocrit, PLT = platelet, LYM = absolute lymphocyte, MXD = absolute mixed count, and NEUT = absolute neutrophil count, MCV = mean cell volume, MCH = mean cell haemoglobin, and MCHC = mean cell haemoglobin concentration. UIBC = unsaturated iron binding capacity, sTfR = serum transferrin receptor, Vit B_{12} = vitamin B_{12}, TIBC = total iron binding capacity, TS = transferrin saturation, BMI = body mass index.

Table 4:15: Haematological and nutritional parameters among the pregnant women in respect of educational level attained in the 3rd trimester.

	Secondary (n=18)	Tertiary (n=50)	
Parameters	**Mean±SD**	**Mean±SD**	**p-value**
Hb(g/dl)	10.94 ± 0.99	11.25 ± 1.13	0.525
PCV (%)	32.89 ± 2.51	33.35 ± 2.26	0.863
MCV (fl)	86.00±9.00	87.31±10.17	0.528
MCH(pg)	28.67±3.43	29.29±3.24	0.737
MCHC(g/dl)	33.39±2.09	33.68±272	0.006
WBC (x10^3/l)	6.61±2.00	7.24±2.29	0.204
LYM(X10^3/l)	1.72±0.57	1.80±0.53	0.460
MXD (X10^3/l)	0.88±0.57	0.81±0.42	0.690
NEUT(X10^3/l)	4.28±1.70	5.00±2.03	0.186
PLT (X10^3/l)	183.33±45.66	217.74±66.83	0.831
Total protein (g/dl)	59.33 ± 4.76	60.94 ± 3.75	0.293
Albumin (g/l)	23.28 ± 2.37	24.70 ± 3.28	0.470
Ferritin (ng/ml)	30.33 ±20.04	30.24 ±20.73	0.829
sTfR($nmol/ml$)	23.61 ± 7.13	23.60 ±6.38	0.921
Folate (ng/ml)	16.94 ± 6.72	21.28 ± 7.93	0.391
Vit B_{12} (pg/ml)	766.00 ± 6383.93	675.46 ± 455.19	0.497
Serum iron (g/dl)	118.28 ± 21.64	122.66 ±20.50	0.764
UIBC (g/dl)	369.44 ± 40.92	380.74 ± 49.77	0.121
TIBC (g/dl)	487.89 ±45.11	503.46 ± 56.49	0.373
TS (%)	24.11 ± 405	24.46 ±3.58	0.620
BMI (kg/m^2)	31.89 ± 5.30	30.06 ± 5.06	0.785

WBC = White blood cell count, Hb = haemoglobin, PCV = haematocrit, PLT = platelet, LYM = absolute lymphocyte, MXD = absolute mixed count, and NEUT = absolute neutrophil count, MCV = mean cell volume, MCH = mean cell haemoglobin, and MCHC = mean cell haemoglobin concentration. Key: P<0.05 is significant, UIBC = unsaturated iron binding capacity, sTfR = serum transferrin receptor, Vit B_{12} = vitamin B_{12}, TIBC = total iron binding capacity, TS = transferrin saturation, BMI = body mass index.

Table 4.16: Haematological and nutritional parameters among the pregnant women in respect of parity in the 3rd trimester.

	Once (n=30)	More than once (n=38)	
Parameters	**Mean±SD**	**Mean±SD**	**p-value**
Hb(g/dl)	11.27 ± 1.23	11.09 ± 1.00	0.113
PCV (%)	33.60 ± 2.28	32.94 ± 2.34	0.416
MCV (fl)	90.53 ±11.43	84.14±7.33	0.002
MCH(pg)	29.91±3.75	28.51±2.92	0.230
MCHC(g/dl)	33.13±2.86	33.97±2.27	0.016
WBC (x10^3/l)	6.63±1.97	7.42±2.36	0.432
LYM(X10^3/l)	1.63±0.56	1.89±0.51	0.026
MXD (X10^3/l)	0.82±0.37	0.83±0.47	0.336
NEUT(X10^3/l)	4.57±1.57	5.00±2.23	0.130
PLT (X10^3/l)	218.67±78.66	200.71±47.97	0.783
Total protein (g/dl)	60.03 ± 3.14	60.89 ± 4.68	0.070
Albumin (g/l)	24.40 ± 2.83	24.26 ± 3.36	0.963
Ferritin (ng/ml)	34.00 ±2.26	27.32 ±19.48	0.369
sTfR($nmol/ml$)	24.77 ± 6.71	22.68 ±6.93	0.830
Folate (ng/ml)	23.03 ± 6.08	17.84 ± 8.34	0.167
Vit B_{12} (pg/ml)	628.90 ±275.01	755.11 ± 631.19	0.105
Serum iron (g/dl)	123.20 ± 20.93	120.16±20.77	0.531
UIBC (g/dl)	382.73 ± 53.61	373.82 ± 45.51	0.090
TIBC (g/dl)	506.03 ±60.14	494 .05±48.46	0.286
TS (%)	24.47 ± 3.82	24.29 ±3.63	0.601
BMI (kg/m^2)	29.47 ± 3.63	31.39 ± 6.00	0.045

WBC = White blood cell count, Hb = haemoglobin, PCV = haematocrit, PLT = platelet, LYM = absolute lymphocyte, MXD = absolute mixed count, and NEUT = absolute neutrophil count, MCV = mean cell volume, MCH = mean cell haemoglobin, and MCHC = mean cell haemoglobin concentration. Key: P<0.05 is significant, UIBC = unsaturated iron binding capacity, sTfR = serum transferrin receptor, Vit B_{12} = vitamin B_{12}, TIBC = total iron binding capacity, TS = transferrin saturation, BMI = body mass index.

Table 4.17 Haematological and nutritional indices among the pregnant women in respect of employed and unemployed in the 3rd trimester.

	Employed (n=38)	Unemployed (n=30)	
Parameters	**Mean±SD**	**Mean±SD**	**p-value**
Hb(g/dl)	11.23 ± 1.25	11.10 ± 0.88	0.121
PCV (%)	33.36 ± 2.23	33.07 ± 2.46	0.721
MCV (fl)	88.39±10.56	85.15±8.56	0.135
MCH(pg)	29.47±3.46	28.70±3.03	0.287
MCHC(g/dl)	33.33±2.58	33.95±2.53	0.931
WBC (x10^3/μl)	7.13±2.32	7.00±2.11	0.442
LYM(X10^3/μl)	1.71±0.56	1.87±0.50	0.078
MXD (X10^3/μl)	0.79±0.51	0.87±0.29	0.004
NEUT(X10^3/μl)	4.97±2.11	4.60±1.77	0.573
PLT (X10^3/μl)	223.95±71.74	189.23±45.23	0.563
Total protein (g/dl)	60.05 ± 3.65	61.10 ± 4.54	0.437
Albumin (g/l)	23.97 ± 2.63	24.77 ± 3.63	0.336
Ferritin (ng/ml)	35.24 ±22.05	23.97 ±16.38	0.159
sTfR($nmol/ml$)	22.92 ± 7.06	24.47 ±6.62	0.689
Folate (ng/ml)	21.21 ± 8.05	18.77 ± 7.43	0.362
Vit B_{12} (pg/ml)	772.03 ±646.88	670.80 ± 240.28	0.048
Serum iron ($\mu g/dl$)	121.92 ± 20.38	120.97±21.59	0.500
UIBC ($\mu g/dl$)	373.87 ± 49.06	382.67 ± 45.93	0.525
TIBC(µg/dl)	495.79 ±60.28	503 .83±44.98	0.080
TS (%)	24.58 ± 2.92	24.10 ±4.51	0.003
BMI (kg/m^2)	31.68 ± 4.85	29.10 ± 5.24	0.762

WBC = White blood cell count, Hb = haemoglobin, PCV = haematocrit, PLT = platelet, LYM = absolute lymphocyte, MXD = absolute mixed count, and NEUT = absolute neutrophil count, MCV = mean cell volume, MCH = mean cell haemoglobin, and MCHC = mean cell haemoglobin concentration. Key: P<0.05 is significant, UIBC = unsaturated iron binding capacity, sTfR = serum transferrin receptor, Vit B_{12} = vitamin B_{12}, TIBC = total iron binding capacity, TS = transferrin saturation, BMI = body mass index.

4.18: The proportion of the study participants to the cutoff value for some selected variables across the trimesters.

Table 4.18 shows the distribution of the study participants to the cutoff value for some selected variables across the trimesters. The proportion of the study participants with anaemia (haemoglobin level <11g/dl) in the 1st, 2nd, and 3rd trimesters were 26(36.11%), 28 (40.58%), and 18 (26.47%) respectively. For total protein, the proportion of the study participants below the cutoff value was observed to increase across the trimesters from 11(15.28%), 25(36.23%0 and 62(91.18%) in the 1st, 2nd, and 3rd, trimesters respectively. Same was observed for albumin levels with 14(19.44%), 51(73.91%) and 67(98.53%) in the 1st, 2nd, and 3rd trimesters respectively. Only a minor proportion of the study participants had folate concentrations below the cutoff value in the 1st, and 2nd, trimesters 1 (1.39%) and 1(1.45%) respectively. No folate deficiency was observed in the third trimester. The proportion of the study participants with Vitamin B_{12} concentration below the cutoff value (200ng/ml), were 2(2.78%), 2(2.9%) and 5(7.35%) representing 1st, 2nd, and 3rd trimesters respectively. Serum ferritin concentration below the cutoff value observed among the study participants across the trimesters were 4(5.56%), 10(14.49%) and 8(11.76%) respectively.

Table 4.18: The proportion of the study participants to the cutoff value for some selected variables across the trimesters.

	1st Trimester	2nd Trimester	3rd Trimester
Variable	**Frequency (%) (n=72)**	**Frequency (%) (n=69)**	**Frequency (%) (n=68)**
Anaemic status [a]			
Anemic	26(36.11)	28(40.58)	18(26.47)
Normal	46(63.89)	41(59.42)	50(73.53)
Total protein status [b]			
Abnormal	11(15.28)	25(36.23)	62(91.18)
Normal	61(84.72)	44(63.77)	6(8.82)
Albumin status [c]			
Abnormal	14(19.44)	51(73.91)	67(98.53)
Normal	58(80.56)	18(26.09)	1(1.47)
Folate status [d]			
Abnormal	1(1.39)	1(1.45)	0(0.00)
Normal	71(98.61)	68(98.55)	68(100.00)
VIT B_{12} status [e]			
Abnormal	2(2.78)	2(2.90)	5(7.35)
Normal	70(97.22)	67(97.10)	63(92.65)
Ferritin status[f]			
Abnormal	4(5.56)	10(14.49)	8(11.76)
Normal	68(94.44)	59(85.51)	60(88.24)

a= Determined using Hb cut off point of 11g/ dl
b= Determined using total protein cut off point of 66g/l
c = Determined using albumin cut off point of 35g/l
d = Determined using folate cut off point of 3.2ng/ml
e = Determined using vit B_{12} cut off point of 200pg/ml
f = Determined using ferritin cut off point of 15ng/ml
g = P<0.01 is significant.

4.19 represents the Pearson's correlation coefficient (r) analysis of ferritin with total protein, albumin, folate, vitamin B_{12}, and body mass index of non pregnant and pregnant women across the trimesters.

Significant positive correlations was found between ferritin and albumin in the pregnant women at 2nd trimester (r=0.460, p=0.001), and ferritin with body mass index (BMI) at 1st trimester (r=0.251, p=0.034) and with vitamin B_{12} in the nonpregnant women (r=0.369, p=0.008).

4.20 shows the Pearson's correlation coefficient (r) analysis of ferritn with serum iron, serum transferrin receptors, total iron binding capacity, unsaturated iron binding capacity and transferrin saturation in the 1st, 2nd, and 3rd trimesters of pregnancy and in the non pregnant women.

As depicted in table 4.20, significant negative correlations were found between ferritin and serum iron in the 2nd trimester (r = -0.300, p = 0.012) and also between ferritin and transferrin saturation in the 2nd trimester (r = -0.306, p = 0.011).

4.21 shows the Pearson's coorelation coefficient (r) analysis of serum iron and TIBC, UIBC, TS% and ferritin during the 1st, 2nd, and 3rd trimesters of pregnancy and in non pregnant women.

Significant positive correlations were found between serum iron and TIBC in the 1st, 2nd, 3rd trimesters and in the non pregnant women (r = 0.355, p = 0.002) (r = 0.315, p = 0.008), (r = 0.479, p = 0.000), and (r = 0.565, p = 0.000) respectively. Serum iron also showed a negative correlation with UIBC in the 2nd trimester (r = 0.940, p = 0.000) and a significant positive correlations with TS% in the 1st, 2nd, 3rd, and in the non pregnant women (r = 0.940, p = 0.000), (r = 0.977, p = 0. 000), (r = 0.780, p = 0.000) and (r = 0.906, p = 0.000).

Table 4:19 Coefficient of correlation between ferritin and total protein, albumin, folate, vitamin B12 and BMI (in each case) during the 1st, 2nd & 3rd trimesters of pregnancy and in non pregnant women.

	Trimester							
	1st (n=72)		2nd (n=69)		3rd (n=68)		Non Pregnant (n=50)	
Variable	**r**	**P**	**r**	**p**	**r**	**p**	**r**	**P**
Total Protein (g/dl)	0.105	.382	-.026	.834	-.014	.991	-.032	.827
Albumin (g/l)	.184	.121	.460**	.000	.162	.188	.235	.100
Folate (ng/ml)	-.062	.607	.113	.356	.155	.206	.137	.343
Vit B12 (pg/ml)	.228	.054	-.078	.527	.130	.291	.369**	.008
BMI (kg/m^2)	.251*	.034	-.099	.418	.033	.791	.002	.989

Key** means significant at 0.01 level
Key* means significant at 0.05 level
BMI-Body mass index

Table 4.20: Correlations between ferritin and iron parameters (TIBC, UIBC, serum iron and percent transferrin saturation) during 1st, 2nd & 3rd trimesters of pregnancy and non pregnant women.

	Trimester							
	1st (n=72)		2nd (n=69)		3rd (n=68)		Non Pregnant (n=50)	
Variable	**r**	**P**	**r**	**p**	**r**	**p**	**r**	**P**
Serurn iron(g/dl)	-.143	.230	-.300*	.012	-.092	.455	-.148	.305
sTfR (nmol/ml)	.100	.403	.173	.155	-.025	.839	-.126	.382
UIBC (g/dl)	.118	.325	.083	.488	-.175	.157	.042	.773
TIBC g/dl)	.062	.603	.083	.500	.187	.127	.042	.773
TS (%)	-.166	.164	-.306*	.011	.020	.870	-.135	.351

Key** correlation significant at 0.01 level
Key* correlation significant at 0.05 level
TIBC=Total iron binding capacity
UIBC=Unsaturated iron binding capacity
TS=Transferrin saturation
sTfR=Serum transferrin receptors

Table 4:21 Coefficient of correlations between serum iron and TIBC, UIBC, ferritin and percent transferrin saturation during 1st ,2nd & 3rd trimesters of pregnancy and non pregnant women.

	Trimester							
	1st (n=72)		2nd (n=69)		3rd (n=68)		Non Pregnant (n=50)	
Variable	**R**	**P**	**r**	**p**	**r**	**p**	**r**	**P**
Ferritin (ng/ml)	-.143	.230	-.300	.012	-.092	.455	-.148	.305
TIBC (g/dl)	.355**	.002	.315**	.008	.479*	.000	.565**	.000
UIBC (g/dl)	-.213	.072	-.343**	.004	.108	.380	-.059	.683
TS (%)	.940**	.000	.977**	.000	.780**	.000	.906**	.000
sTfR (nmol/ml)	-.003	.980	-.029	.814	-.107	.385	.154	.286

Key** means significant at 0.01 level
Key* means significant at 0.05 level
TIBC –Total iron binding capacity
UIBC-Unsaturated iron binding capacity
TS- Transferrin saturation
sTfR-Serum transferrin receptors

CHAPTER FIVE

5.0 DISCUSSION

This longitudinal study examined some nutritional indices such as vitamin B_{12}, folate, iron levels and some haematologic parameters in normal pregnant women attending antenatal care at the University of Calabar Teaching Hospital in Calabar, Cross River State. The demographic characteristics of the study participants such as age, education, parity, household size and average monthly income were analyzed and used to evaluate the relationship with the nutritional indices measured. In this study, 52.78% of the pregnant women are within the age group 18 to 29 years while 47.22% were within 30 and 41 years. This implies that teenage pregnancy was not recorded and as such its consequencies could be far fetched.

Low educational attainment is one of the potential risk factors associated with decrease in the levels of nutritional indices in pregnancy. When considering the educational level, more than half of the study participants (73.61%) had attained at least tertiary level of education while 26.39% attained up to secondary education. Sahoo and Panda, (2006) recorded 9.5% illiterate pregnant mothers while this present work recorded no illiterate pregnant mother among the respondents. The educational level is important in order to acquire knowledge about nutritional requirements (Sahoo and Panda, 2006) which include: to take adequate nutrients during pregnancy by indulging in a healthy lifestyle, improve access to medical care and health care services, reduce use of harmful substances during pregnancy, taking folic acid and other supplements which will reduce high risk births (Zerfu and Ayele, 2013). Higher percentage of the pregnant women in this study having attained tertiary education may have contributed to early and regular visits to the ante natal clinics by both primigravidae and multigravidae, receiving antenatal care during pregnancy and participation in nutritional education programs which were conducted at the clinic.

On the other hand, the proportion of the study participants that were unemployed (43.06%) even after attaining tertiary education should be seen as a barrier to achieving and maintaining an adequate nutritional status during pregnancy. Due to the alarming population of the low income earners recorded among this group of pregnant women, maternal micronutrient deficiencies resulting from the expense of, and/or lack of access to foods rich in multiple micronutrients could be possible since income level is related to poor pregnancy outcome such as low birthweight (Kramer *et al*., 2000).

The level of unemployment recorded among the study participants could lead to pregnancy related anxiety and may contribute to maternal stressors capable of affecting the levels of micronutrients. To the working class among them, strenuous physical work, and work related stress could contribute to maternal stressors (Hobel and Culhane, 2003) and these are capable of giving rise to reduced levels of micronutrients.

This present work disagrees with the findings of Ogbodo *et al.*, 2012 which recorded that only primigravidae (women who are in their first pregnancy) enroll for antenatal care in their first trimester. The study recorded pregnant women in more than once parity who enrolled as participants, even in the midst of harsh or adverse socioeconomic environment, and with the high level of unemployment (43.06%) recorded among the study participants in this study.

This present work recorded decrease in total protein across the trimesters. The decrease was statistically significant in the 3rd trimester (p=0.01) when compared to the 1st trimester and the non pregnant women. The findings from this work show that total protein level decreases with increase in gestational age. This could be due to changes in protein metabolism which occur in early pregnancy presumably in response to pregnancy related hormones (Kalhan, 2000). The progressive fall or decrease in serum protein could be attributed to the amount of protein deposited in maternal and fetal tissues, with non significant deposition during the first trimester, gradually increasing during the second trimester, and with most occurring in the third trimester (Butte and King, 2005; Kathleen and Drora, 2010). Moreover, protein needs are increased as early as 16 weeks of gestation (Elango and Ball, 2016), although it was previously thought that the demand for protein would be low initially and increase substantially only by late pregnancy (King, 2000). Also the extra protein needed to support the formation of red blood cells and the circulating proteins especially as women's blood volume increases in pregnancy may contribute to the reduction of serum protein levels across the trimesters. It is suggested that the mother stores protein in muscles during the second trimester which is drawn upon in the third trimester to support the growth of the foetus (Duggleby and Jackson, 2001; 2002).

Similarly, result of the albumin level of the study participants showed significant decrease across the trimesters (p=0.01) as the albumin levels decreased with increase in gestational age. A study carried out by Ogbodo *et al.*, 2012 recorded decrease in protein and albumin levels among pregnant women in a rural area of Ebonyi State, Nigeria. Hytten (1991) showed a steady decline in serum total protein and albumin levels across the trimesters (Hytten, 1991). Apparently, decreases in total protein and albumin levels seen amongst the study

participants with increasing gestation may be a mechanism to ensure a foetal adequate nutrient delivery. However, the progressive fall or decrease in albumin level across the trimesters is most likely due to hypervolemia (which is consistent with pregnancy) that causes dilution effects. Moreover, because of its molecular weight, albumin is the fraction that is usually lost during proteinuria in pregnancy. Thus, hypoalbuminemia in pregnancy may not necessarily be only due to decrease in production but also dilution (due to increased volume) and increased loss in urine (proteinuria). These alteration or reduction in the serum protein and albumin across the trimesters as pregnancy progressed may result in alterations to the immune status of pregnant women though this is required to enable mothers tolerate genetically different fetal tissues during pregnancy. The reduction in the serum protein and albumin levels may also increase susceptibility of the pregnant women to infections (Yip *et al.*, 2006). Immune proteins are particularly significant in their role in preventing immune-related complications including infections and infestations. Interestingly, the development of immunity is said to increase with parity (Riley *et al.*, 1989; Rasheed *et al.*, 1993; Rogerson *et al.*, 2007), therefore, a low serum protein means low immunity and is a threat to primigravidae and secundigravidae.

The present work recorded no significant change across the trimesters but decrease in vitamin B_{12} level when compared to the value of the non pregnant women. The decline or decrease in vitamin B_{12} is in agreement with the findings of Fernandes- Costa and Metz, (1982) and Karim *et al.*, (2010) who recorded a steady decline throughout pregnancy commencing from 1st trimester (Fernandes- Costa and Metz, 1982; Karim *et al.*, 2010). Bruinse and van den Berg, (1995), Pardo *et al.*, (2000), Koebnick *et al.*, (2002) and Murphy *et al.*, (2007) also observed reductions in vitamin B_{12} concentration throughout the course of normal pregnancy. Morkbak and colleagues, in a longitudinal study, observed that serum vitamin B_{12} levels decreased over the course of pregnancy from 18weeks gestation up to the time of delivery (Morkbak *et al*., 2007). The decrease in vitamin B_{12} concentration when compared to the non pregnant is due to the rapid cell multiplication resulting from uterine enlargement, placental development and foetal growth. This physiological normal decline in serum vitamin B_{12} concentrations is also thought to be due to several other factors such as haemodilution, hormone fluctuations, impaired renal function or altered concentration of binding proteins (Koebnick *et al.*, 2002; Guerra-Shinohara *et al.*, 2004; Milman *et al.*, 2006) or active transport of vitamin B_{12} across the placenta (Obeid *et al.*, 2006). The fluctuation observed in the serum vitamin B_{12} concentration in the 3rd trimester could be attributed to the reduction in maternal plasma volume during the 3rd trimester which is common in pregnancy.

The present study revealed decreased concentration of serum folate from 1st to 2nd trimester when compared to the non pregnant control but was increased in the 3rd trimester. This decrease in the 2nd trimester could be due to haemodilution and hormonal changes. The increase in folate concentration in the 3rd trimester could still be attributed to the reduction in maternal plasma volume during the 3rd trimester. Several previous studies reported that serum folate concentration slightly decreases during pregnancy with recovery after delivery (Bruinse and van den Berg, 1995; Rolf *et al.*, 2001). This present work does not agree with the findings of Karim *et al.*, (2010), which revealed significant higher folate concentrations in pregnant women in all the three trimesters (Karim *et al.*, 2010) which could be diet or supplements intake related.

The body mass index (BMI) of the study participants depicts that there was weight gain across the trimesters which is common in pregnancy. The increase in weight across the trimesters was not significant but only when compared to the non-pregnant women. The total amount of weight gain during pregnancy is determined by many factors. Aside from physiological factors, psychological, behaviuoral, family, social, cultural and environmental factors can have an impact on gestational weight gain. Poor eating habits and sedentary behaviuors shaped during childhood and adolescence may be carried into young adulthood and continued into pregnancy, with the potential to affect gestational weight gain indirectly. But since excessive gestational weight gain has been shown to be strongly associated with maternal-foetal complications such as gestational diabetes mellitus, hypertensive pregnancy disorders, mansonia and low birth weight (Olson *et al.,* 2004; Stotland *et al.,* 2005; Mohanty *et al.*, 2006), therefore, the context of 'eating down' during pregnancy to avoid the hazard of difficult labor is necessary.

This present study revealed a significant decrease in packed cell volume (PCV) values in the 1st, 2nd, 3rd trimesters when compared to the non pregnant control. Similar result was observed by Wulsa and his colleagues (Wulsa *et al.*, 2015), who reported that PCV values showed statistical significant decrease in pregnant women across the trimesters when compared to the controls with ($p=0.01$). This decrease in PCV values could be due to marked increase in plasma volume associated with normal pregnancy causing dilution of many circulating factors and cells resulting in physiological anaemia (Salawu and Durosinmi, 2000; James *et al.,* 2008; Imoru and Emeribe, 2009). The PCV value in the 3rd trimester was slightly increased than in the 1st and 2nd trimesters. The drop in PCV by the late second trimester and thereafter stabilizes in the third trimester, when there is a reduction in maternal

plasma volume is due to an increase in the levels of atrial natriuretic peptide (Barriga *et al.*, 1994; Ajzenberg *et al.*, 1998).

This present study recorded a decline in haemoglobin (Hb) concentration in all the trimesters though more decreased in the 2nd trimester among the study participants. Akinbami *et al.*, (2013) found a progressive decline in Hb concentration from 1st to 3rd trimester. This also is in line with the findings of other studies (James *et al.*, 2008, Imam, 2008; Pilsczek, 2008). The decline in Hb concentration may be due to increased demand for iron as pregnancy progresses (Akinbami *et al.*, 2013). More iron is required to meet the expansion of maternal haemoglobin mass and the needs of foetal growth. The additional progesterone and oestrogen that are secreted by the placenta during pregnancy cause a release of rennin from the kidneys (Akinbami *et al.*, 2013). During pregnancy, plasma rennin activity tends to increase and atrial natriuretic peptide levels tend to reduce though slightly. This suggests that in pregnant state, the elevation in plasma volume is in response from systemic vasodilation rather than the actual blood volume expansion (Barriga *et al.*, 1994; Ajzenberg *et al.*, 1998). The drop in haemoglobin is typically by 1–2 g/dL by the late second trimester and stabilizes thereafter in the third trimester, when there is a reduction in maternal plasma volume owing to an increase in levels of atrial natriuretic peptide (Barriga *et al.*, 1994; Ajzenberg *et al.*, 1998).

Data in the present study showed that there is a significant decrease in mean cell volume (MCV) of the pregnant women in all the trimesters when compared to the non pregnant women. This is in agreement with the findings of Akinbami *et al.*, (2013) who reported that MCV declined from the 1st to the 3rd trimester. But Azab *et al.*, (2017) reported only a decline in 2nd trimester. This finding may be a reflection of iron deficiency anaemia (Akinbami *et al.*, 2013). Crocker and colleagues postulated that MCV does not change significantly during pregnancy and haemoglobin of 9g/dl in association with a MCV of 84fl probably indicates co-existent of iron deficiency or some other pathology (Crocker *et al.*, 2000).

Mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) did not show any significant difference in the pregnant women when compared to the non pregnant women. Chaudhari and Bodat, (2015) also recorded no significant difference in the mean MCH and MCHC among the pregnant women (Chaudhari and Bodat, 2015). The present study agrees with the findings of Akinbami *et al.*, (2013) who reported that MCH remained relatively stable through all the trimesters but MCHC dropped only in the 3rd trimester (Akinbami *et al.*, 2013).

In the present study, white blood cell (WBC) count was significantly increased through all the trimesters when compared to the non pregnant women. WBC's are responsible for body defense during pregnancy. The increase observed in WBC count through all the trimesters in this present study is consistent with the findings of Akinbami *et al.*, 2013). Other previous studies includes Onwukeme and Uguru, 1990; Akingbola *et al.*, 2006; Oke *et al.*, 2011; Osonuga *et al.*, 2011) who asserted that an increased WBC count rises early in pregnancy and remain elevated throughout pregnancy. Pain, nausea, vomiting and anxiety have been reported to cause leucocytosis (Akinbami *et al.*, 2013). A rise in WBC count is not a reliable indicator of infection in subclinical chorioamnionitis: rather clinical methods of detection such as maternal pyrexia, offensive vaginal discharge, and foetal tachycardia are better indicators, especially of preterm labour and membrane rupture (Akinbami *et al.*, 2013). This may be as a result of the body building the immunity of the foetus and it is achieved by a state of selective immune tolerance, immunosuppression, and immunomodulation in the presence of a strong antimicrobial immunity. There is also down regulation of potentially dangerous T-cell-mediated immune responses, while activating certain components of the innate immune system, such as neutrophils.

Data in the present study showed no statistical difference between the mean neutrophils in both the study participants and the control. Das and his colleagues, made the same observations (Das *et al.*, 2013). In the present study, platelets count exhibited a highly significant decrease in the 2^{nd} trimester when compared to the 1^{st} trimester and a significant decrease in the 2^{nd} and 3^{rd} trimesters when compared to the non pregnant women. Similar results were noticed by Akinbami *et al.*, (2013) and Akingbola *et al.*, (2006) who reported a gradual reduction in platelets count as pregnancy progresses (Akinbami *et al.*, 2013; Akingbola *et al.*, 2006). Abbassi –Ghanavati, (2006) reported that platelets count was slightly lower in pregnant women than in non pregnant women. Due to haemodilution secondary to expansion of plasma volume, the platelets count in normal pregnancies may decrease with most of this decrease occurring during the 3^{rd} trimester (Ballem, 1988; McCrae, 2003). Platelets count may be lower with twin compared with singleton pregnancies, possibly due to greater thrombin generation (Tsunoda *et al.*, 2002). The mechanisms for this are thought to be due to dilution effects and accelerated destruction of platelets passing over the often scarred and damaged trophoblast surface of the placenta (Fay *et al.*, 1983; Akinbami *et al.*, 2013). Although, most cases of thrombocytopaenia in pregnancy are mild with no adverse outcome for mother or baby, occasionally, a low platelets count may be part of a complex disorder with significant morbidity and be rarely life threatening.

Serum ferritin level in the study participants decreased across the trimesters. The fall was markedly observed in the 3rd trimester. This is in agreement with the results reported by other studies (Alper *et al*., 2000; Abel *et al*., 2001; Raza *et al.,* 2011). Serum ferritin usually falls markedly between 12 and 25 weeks of gestation, probably as a result of iron utilisation for expansion of the maternal red blood cell mass (Hytten, 1985; Letsky, 1998). The decrease in ferritin levels as pregnancy progressed from first to second and to third trimesters, appears to follow the same observations made by Horton *et al,* (2013) and others. These could likely be due to haemodilution and increase in erythropoiesis (Talwar and Srivastava, 2006; Horton *et al.,* 2013). Asif *et al*, 2007 and Bhale *et al*, 2013 both reported continous decrease in serum ferritin level while Kumar *et al*, 2017 reported a decrease only in the 2nd trimester. In this study, the consistent drop in serum ferritin levels suggests that the iron stores of the subjects or respondents would have been decreased as reflected in the serum ferritin levels. It was considered that all the pregnant women were taking adequate supplementation of iron as well as on adequate diet. But it cannot be ignored that they might lack regular diets as well as iron supplements which was not strictly followed in this study.

Iron deficiency in pregnancy has been defined by the National Academy of Sciences panel on nutrition and pregnancy (1990) as ferritin levels lower than 12ηg per ml. Ferritin is decreased with iron deficiency anemia and is increased with elevated total body stores of iron. Ferritin is also an acute phase protein, and hyperferritinemia can occur with underlying disease, such as inflammatory disease, neoplasia, liver disease, or haemolytic disease. Ferritin levels are considered the gold standard for the diagnosis of iron-deficiency anemia in pregnancy (Clark, 2009).

The serum iron level in the 1st trimester (89.94 ±23.03) ug/dl to the 2nd trimester (70.30 ±29.98) ug/dl and in 3rd trimester (121.50 ±20.74) ug/dl, showed a significant change. Drop in serum iron level was observed from 1st trimester to 2nd trimester which again rises in the 3rd trimester. The decrease in the serum iron level in the 2nd trimester is in accordance with the findings of earlier studies like Okafor *et al.,* (2016) and Chaudhari *et al.,* (2015). The body"s mechanism to meet the sufficient supply of iron could be the reason for this drop. The blood samples collected in both studies for estimating 1st, 2nd and 3rd trimester serum iron level were from pregnant females at different periods of gestation. While in this study the women were inducted in the 1st trimester and followed through all the trimester. The challenge with the measurement of serum iron levels directly in the blood is the fact that the levels increase immediately being influenced by recently ingested meals and with iron supplementation (patient must stop supplements for 24 hours). This significant increase

observed in the 3rd trimester could be attributed to the accumulated menstrual savings and increased maternal intake of haem and non-haem iron diet since absorption of haem and non-haem iron is known to increase especially in the 3rd trimester (Hallberg, 1994). Most iron transfer to the foetus occurs after 30 weeks of gestation which correspond to the time of peak efficiency of maternal iron absorption (Sakande *et al.*, 2004). Serum iron levels are not helpful by themselves because they vary with time of the day as it demonstrates diurnal variation, with a rise in the morning and fall at night (Tietz *et al.*, 1994) and due to various systemic insults (Favier and Ruffieux, 1983). The serum iron reflects both iron recycling from macrophages and iron absorbed from the diet.

There is a significant decrease in the percent transferrin saturation (TS %) of the pregnant women in the 2nd trimester when compared to the 1st and 3rd trimesters. The drop in transferrin saturation among the study participants could be a consequent of a progressive drop with increase in gestational age in protein and albumin levels recorded among the study participants since transferrin saturation tends to drop when there is not enough protein in the diet. Also, if there is low total body iron (iron deficiency) or trapping of iron in macrophages (anemia of inflammation), percent transferrin saturation tends to be low.

The ratio of serum iron to TIBC (transferrin saturation index or percent) is the most specific indicator of iron deficiency, when it is sufficiently low. The iron saturation (or transferrin saturation TS) of <5% almost always indicates iron deficiency, while levels from 5% to 10% make the diagnosis of iron deficiency possible but not definitive. Saturations over 12% (taken alone) make the diagnosis unlikely. Normal saturations are usually (<16%) for pregnant women. However, the mean ferritin and percent transferrin saturation suggest that a high percentage of the pregnant women experienced iron-status indicator deficiencies in the second and third trimesters (for ferritin only). This present study recorded a statistically significant decrease in unsaturated iron binding capacity (UIBC) in pregnant women in the 3rd trimester when compared to the 1st and 2nd trimesters and the non pregnant women. No significant difference was observed for total iron binding capacity (TIBC) in the pregnant women across the trimesters and when compared to the non pregnant. This is in disagreement with the findings of Lee *et al.*, (2006) which recorded more pronounced increase in UIBC as the pregnancy advanced to term (Lee *et al.*, 2006). Dapper *et al.*, 2006 and Amah-Tariah and colleagues both reported increases in total iron binding capacity and unsaturated iron binding capacity with increase in gestational age (Dapper *et al.*, 2006; Amah-Tariah *et al.*, 2011). The difference in the present study with the other previous studies could be due to differences in methods applied in the measurements and the fact that blood samples collected for estimating

1st, 2nd and 3rd trimesters levels of both UIBC and TIBC were from pregnant females at different periods of gestation.

However, values of unsaturated and total iron binding capacity in the present study showed a pattern opposite that of serum iron concentration: the lowest value for serum iron was observed to correspond with the highest value of unsaturated and total iron binding capacity amongst the pregnant women in the 2nd trimester of pregnancy.

The present study recorded 1(1.39%) and 1(1.45%) of the participants with folate concentration below the WHO recommended cutoff level of 3.2ng/ml in the 1st and 2nd trimesters respectively but no participant had folate level below the recommended cutoff value in the third trimester. VanderJagt *et al.*, (2009) revealed that 4% of the 98 pregnant women had a serum folate concentration less than the cutoff for serum folate which is 3.2ng/ml. Karim and colleagues recorded no folate deficiency in all the trimesters in the Pakistinian pregnant women (Karim *et al.,* 2010). This present longitudinal study is in partial conformity with the findings of Karim and his colleagues since they worked with pregnant women in different trimesters of pregnancy. There may be two reasons for the observed increase of folate concentration in the present study which resulted in no participant with folate concentration below the recommended cutoff level: one may be dietary intake since the people in this locality use different types of vegetables in their meals. Another reason may be folate supplementation in pregnancy. The present study therefore does not agree with the works of Karaoglu *et al.,* (2010) which recorded higher folate deficiency than iron deficiency in pregnancy.

The findings from the present study showed 2(2.78%), 2(2.90%), 5(7.35%) of the pregnant women had vitamin B_{12} below the WHO recommended cutoff value of 200ng/ml. VanderJagt and coworkers (2007) found out that 9% of the 146 pregnant women had a serum vitamin B_{12} concentration below the 148pmol/L (200ng/ml) which is the cutoff for vitamin B_{12} deficiency (Carmel *et al,* 2003). VanderJagt *et al.*, (2009) in a subsequent study documented that 12% of the 98 pregnant women had vitamin B_{12} levels in the deficiency range and in a cross sectional study of 143 pregnant women in Jos (Nigeria), VanderJagt *et al.*, (2011) revealed that 36% of the pregnant women were vitamin B_{12} deficient. Achebe and Gafter-Gvili, (2017), recorded that cobalamin deficiency in pregnancy is far less common than folate deficiency owing to the relatively large amounts of cobalamin that are stored in the human body. But the present study recorded more percentage of the pregnant women with vitamin B_{12} below the cut off value of 200ng/ml than folate. The disparity in the results could

be attributed to the differences in the study design, since they recruited pregnant women in different trimesters of pregnancy while the present study is a longitudinal or a follow up study. Therefore, World Health Organization (WHO) recommend a higher daily allowance of cobalamin in pregnant women than in nonpregnant women (2.6 vs 2.4 mg per day) to support fetal neurologic development (WHO, 2004).

Significant negative correlations was found between ferritin and serum iron in the 2^{nd} trimester (r = -0.300, p = 0.012) and also between ferritin and transferrin saturation in the 2^{nd} trimester (r = -0.306, p = 0.011). Significant positive correlations was found between ferritin and albumin in the pregnant women in the 2^{nd} trimester (r =0.460, p = 0.001), and ferritin with body mass index (BMI) in the 1^{st} trimester (r = 0.251, p = 0.034) and with vitamin B_{12} in the non pregnant women (r = 0.369, p = 0.008). Previous studies have shown these relationships between serum iron and other iron parameters. Raza and his colleagues showed a correlation between serum iron and TIBC, UIBC and percent transferrin saturation (Raza *et al.*, 2011). This confirms an inverse relationship between serum iron and TIBC and UIBC which states that while serum iron decreases, TIBC and UIBC increases and vice versa.
Noteworthy are the demographic characteristics showing an association in both the 2^{nd} and 3^{rd} trimesters with some of the nutritional indices measured in this present study. The MXD, folate and vitamin B_{12} concentrations of the primigravidae were significantly increased (p=0.025, p=0.010, p= 0.011) respectively than those of more than one parity (table 4.13). The MCV, LYM and BMI of the primigravidae is significantly lower (p =0.045) than in more than once parity (table 4.16). Moreover, vitamin B_{12} concentration of employed pregnant women in the 3^{rd} trimester, was significantly higher (p =0.048) than in the unemployed (table 4.17). Interestingly, the percent transferrin saturation of the employed was statistically increased than that of the unemployed participants in the 3^{rd} trimester (table 4.29). These could be due to the fact that multi parity, poor socio economic and educational statuses are the principal reasons for a poor pregnancy outcome in our population. Isah, *et al.*, (1985) reported 37% and 52% in elite and non-elite pregnant women, coming down with adverse pregnancy outcomes may be due to the commercialization of ante-natal service in the present day as opposed to the free services rendered in the past. Thus women of all socio-economic background attended the antenatal care (ANC) in the past but now only educated and well to do women attend ANC regularly. Lack of employment opportunities, will result in lack of ability of women to command resources and make independent decisions about their fertility, their reproductive health and healthcare and hence, will impact negatively on the maternal nutrition.

5.1 Conclusion

The results of the study showed that folate level was decreased in the 2nd trimester when compared to the 1st and 3rd trimesters. The serum iron level in the 3rd trimester was increased when compared to the 1st and 2nd trimesters. Total protein and albumin levels of the pregnant women decreased with increase in gestational age. Haemoglobin, packed cell volume, and mean cell volume did not show any significant change across the trimesters but were found to be decreased when compared to the non pregnant women. While this group of pregnant women appears to have decrease in ferritin concentrations across the trimesters, total iron binding capacity (TIBC), vitamin B_{12} and serum transferrin receptors (sTfR) did not show any significant change across the trimesters.

Recommendations

Maternal/perinatal morbidity and mortality has remained unacceptably high. This is due to the levels of maternal nutritional indices are not encouraging. The government or the non-governmental agencies should channel energies and resources to improve the appalling situation, in both the rural and the urban areas in order to prevent maternal malnutrition. The government should also promote and foster the development of environments that support a healthy lifestyle.

Iron supplementation may be necessary only if iron deficiency anaemia is diagnosed since prophylactic supplementation may increase risk when the mother does not have iron deficiency or iron deficiency anaemia. This therefore calls for assessment of iron stores at each stage and condition of pregnancy as part of the routine tests to be carried out on the pregnant women.

It is necessary to propose increased intake of vitamin B_{12} during pregnancy either through supplementation or via fortified foods since the placenta prefers to transport directly the ingested vitamin B_{12} than the vitamin B_{12} liver stores to the foetus.

Intake of protein diets should be encouraged during pregnancy.

Limitations

There are limitations with this study. First, the sample size of 72 pregnant mothers in the first to 69 in the second, and to 68 in the third trimester was due to factors such as loss of pregnancy (ectopic pregnancy) and preeclampsia.

Examination of stool sample was not done for the analysis of the presence of intestinal parasites since infection with some intestinal parasites is known to affect vitamin B_{12} absorption.

Furthermore, a 24 h dietary recall was not taken into cognizance in this study though this may yield an accurate representation of the nutrient status of the study participants.These short comings will be taken care of in future studies.

Contributions to knowledge

This is the first longitudinal study has been done in this locality to evaluate the nutritional indices (vitamin B_{12}, folate, iron levels and some haematologic parameters) in normal pregnant women. Hence, the research work has enlightened Clinicians on the need to always check the levels of these parameters throughout the course of pregnancy in order to monitor and predict poor pregnancy outcome.

The research work has also enlightened the pregnant women on the need for early antenatal care in order to detect high risk pregnancy and thus prevent poor pregnancy outcomes.

The present study has also provided reason for pregnant women attending antenatal clinics to benefit from vitamin B_{12} supplementation just as they are routinely given iron and folate supplements when they register to avoid anaemia**.**

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APPENDIX I

CONSENT FORM

I have fully explained this research to…….and given sufficient information including risk and benefits to make an informed decision.

DATE:.....

SIGNATURE: .....

NAME: .....

STATEMENT OF PERSON GIVING CONSENT

I have read the description of the research or have had it translated with a language I understand. I have also talked with the scientist. I understand that my participation is voluntary.

I know enough about the purpose, methods, risks and benefits of the research to judge that I want to take in it. I understand that I may freely stop being part of this study at any time.

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

Participants Signature: ..... Thumbprint:.....

Date: .....

Witness Signature: ..... Thumbprint: .....

Date: .....

APPENDIX II

QUESTIONNAIRE

IDENTIFIER NUMBER

AGE: 18-29years [ ] 30-39years [ ] 40years and above [ ]

HEIGHT: .....

WEIGHT:.....

BODY MASS INDEX: .....

BLOOD PRESSURE: .....

EDUCATION: PRIMARY [ ] SECONDARY [ ] TERTIARY [ ]

OCCUPATION: PUBLIC/CIVIL SERVANT [ ] TRADER [ ]

HOUSEWIFE [ ]

SIZE OF HOME: .....

MONTHLY INCOME: .....

WHICH TRIMESTER ARE YOU? 1 – 3 months [ ] 4 – 6 months [ ]

7- 9 months [ ]

PARITY: .....

ANY HISTORY OF CHRONIC OR COMMUNICABLE DISEASE?.....

HOW OFTEN DO YOU TAKE VEGETALES AND FRUITS? ALTERNATE DAYS [ ] ONCE DAILY [ ] TWICE DAILY [ ]

HAVE YOU HAD MISCARRIAGE BEFORE? .....

DO YOU FAST ANYTIME/ANYDAY? .....

DO YOU TAKE THREE SQUARE MEAL DAILY? .....

DO YOU TAKE A BALANCED DIET: MEAL TO PROVIDE PROTEIN, FAT, MINERALS, STARCH ETC YES [ ] NO [ ]

HOW OFTEN DO YOU TAKE SNACKS/FAST FOOD? DAILY [ ]

ALTERNATE DAYS [ ]

DO YOU TAKE EGG, MILK ETC? YES [ ] NO [ ]

HOW OFTEN DO YOU TAKE MEAT: BEEF, CHICKEN, TURKEY, SNAILS, FISH ETC: .....

ARE YOU ON IRON SUPPLEMENTS? YES [ ] NO [ ]

ARE YOU ON FOLATE SUPPLEMENTS? YES [ ] NO [ ]