IRON STATUS OF PREGNANT WOMEN AT DIFFERENT TRIMESTER IN NSUKKA, ENUGU STATE

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TITLE PAGE

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A DISSERTATION SUBMITTED IN PARTIAL FULLFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF DEGREE OF MASTER OF SCIENCE (M.Sc.) DEGREE IN NUTRITIONAL BIOCHEMISTRY, DEPARTMENT OF BIOCHEMISTRY, UNIVERSITY OF NIGERIA, NSUKKA

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CERTIFICATION

Uzoigwe Kelechukwu Chinaza, a post graduate student with Registration Number PG/M.Sc/17/00238 in the Department of Biochemistry, faculty of Biological Sciences, University of Nigeria, Nsukka has satisfactorily completed the requirements for course work and research for the award of degree of Master of Science (M.Sc) in Biochemistry (Nutrition). The work contained in this report is original and has not been submitted in part or full for any other diploma or degree of this or any other university.

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PROF B. C. NWANGUMA **PROF H. A ONWUBIKO**

(Supervisor) (Head of Department)

…….

EXTERNAL EXAMINAR

DEDICATION

This work is dedicated to the Almighty God and to my Daddy, late Mr. Kelvin Uzoigwe, my loving mother, Mrs Eucharia C. Uzoigwe, my best friend Ifeanyi Ephraim Uzuegbe and to my entire family.

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ABSTRACT

The aim of this experiment was to determine the prevalence and severity of anaemia in pregnant women in first, second and third trimester using haemoglobin and ferritin concentration as an indicator of anaemia among the subjects. Effect of age, gestational age (trimester) and intake of routine drug on haemoglobin and ferritin concentration of the subjects were also determined. In this study a total of 127 blood samples from pregnant women within the age of 20 – 49 years, in which 6 were in first trimester, 50 in second trimester and 71 in third trimester were collected. From this study, the prevalence of anaemia in the subjects based on haemoglobin concentration below 11 g/dl was 39.4%. Prevalence based on ferritin concentration below 15 µg/l was 27.6%. The result of correlation analysis of haemoglobin concentration on the effect of age shows a negative correlation which is significant ($p < 0.05$) but positively correlated with ferritin concentration although not significant at ($p > 0.05$). Gestational age when correlated with haemoglobin and ferritin concentration shows a negative correlation at ($p > 0.05$). Intake of routine drug when correlated with haemoglobin shows a negative correlation at ($p > 0.05$) but when correlate with ferritin shows a positive correlation, although not significant at ($p > 0.05$). This study reveals that more than half of the subjects were anaemic and their children stands the risk of being born anaemic especially when they are exclusively breast fed. Therefore, public health campaign should be carried out to sensitize pregnant women on the need for early antenatal booking and iron supplementation during pregnancy.

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LIST OF ABBREVIATIONS

DMT1	Divalent Metal Iron Transporter 1
EAR	Estimated Average Requirement
EFSA	European Food Safety Authority
FPN1	Ferroportin 1
GDS	Gastric Delivery System
IREs	Iron Response Elements
IRPs	Iron Regulatory Protein
NTBI	Non – transferrin Bound Iron
PRI	Population Reference Intake
RDAs	Recommended Dietary Allowance
TF	Plasma Transferrin
TFR	Transferrin Receptor
TSAT	Transferrin Saturation

CHAPTER ONE

INTRODUCTION

Iron is a type of mineral found in all cells of the body. Iron is obtained from the diet, enters the body and is carried throughout the bloodstream by a protein called transferrin, which is produced in the liver (Elzahrani, 2012). In the blood iron helps form haemoglobin which is an important protein in red blood cells that helps transport oxygen throughout the body so it can function normally. Iron is considered an important mineral because haemoglobin cannot be made without it (Elzahrani, 2012).

Iron deficiency is the most frequent nutritional deficiency disorder in the world (WHO, 2015). A recent estimate based on World Health Organization (WHO) criteria indicate that around 600-700 million people worldwide have a marked iron deficiency anaemia. Iron deficiency anaemia in pregnancy has been defined by the National Academic of Science Panel on nutrition as ferritin level lower than 15 µg/l while the World Health Organization defined anaemia in pregnancy as haemoglobin level of less than 11 g/dl (Elzahrani, 2012). For non – pregnant women, the World Health Organization (WHO, 2015) defined anaemia as haemoglobin concentration less than 12g/dl and defined iron deficiency anaemia as serum ferritin concentration less than 15 µg/l.

Pre-pregnancy body stores are important because during pregnancy there is a marked physiologic increase in the demands for absorbed iron to expand the woman's red blood cell mass and to secure an adequate iron supply for the function of the placenta and developing foetus (Milman, 2006). To complete a normal pregnancy without taking iron supplements and without developing iron deficiency or iron deficiency anaemia, the woman should have body iron stores at conception of ≥500 mg (WHO, 2015), which corresponds to serum ferritin concentration of 70 – 80 µg/l (Milman, 2006).

The development of iron deficiency anaemia is associated with increased risk of pre-term birth and low birth weight infants. The physiologic importance of storage iron is that it provides a rapidly available supply in the event of blood loss. To achieve iron balance, towards the end of pregnancy, the absorption of 4 – 5 mg/day is necessary. Requirements are higher during periods of rapid growth in early childhood and adolescent. Worldwide, the highest prevalence figures of iron deficiency today are found in infants, children, teenagers, women of childbearing age

and pregnant women. The highest prevalence today is observed in pregnant women and menstruating women (Sachan *et al.*, 2013).

Transfer of iron from mother to foetus occurs mainly in the last trimester of pregnancy. Therefore, during this period a mother's food should contain surplus quantities of iron. During this time, the child is dependent on the iron reserve, receive from the mother during pregnancy. In premature babies, the trans placental transfer of iron might not have taken place. Hence such babies are at risk of iron deficiency (Vasudevan *et al.*, 2011). Haemoglobin concentrations are used to characterize, and serve as iron benchmark for anaemia and iron deficiency anaemia have been provided by the WHO (WHO, 2015). Iron deficiency is commonly caused by the combination of blood loss and insufficient dietary intake. (Milman, 2006).

1.2 Importances of Iron Stores in Pregnancy

Unfortunately, many women begin their pregnancy without having sufficient stores of iron to meet their body's increased demand leading to iron deficiency. It is apparent that iron balance can be maintained in pregnancy only when there are adequate iron stores at the start of pregnancy (Sandstorm, 2001). If a woman routinely eats a diet high in bioavailable iron, a pre-pregnancy iron store of 300 mg is probably sufficient to carry her through pregnancy, although a higher amount of stored iron is needed when the diet is less than optimal (Sandstorm, 2001).

During pregnancy more iron is needed primarily to supply the growing foetus and placenta and to increase the maternal red blood mass (Sloan *et al.*, 2012). The extent to which women of reproductive age can meet their iron requirements during pregnancy has been estimated from studies that calculated iron stores through measurements of serum ferritin concentration and other hematologic indexes (Sloan *et al.*, 2012). Studies have showed that haemoglobin concentration during the last half of pregnancy were found to be higher in iron supplement women than in those with no iron supplement (Cogswell *et al.*, 2003). The higher the haemoglobin concentration as a result of improved iron supply not only increases the oxygen carrying capacity but also provides a buffer against the blood loss that will occur during pregnancy (Cogswell *et al.*, 2003).

1.3 Sources of Dietary Iron

Dietary iron contains both haem and non-haem iron. Both forms of iron are absorbed non-competitively into duodenal and jejunal mucosal cell (Hurrel and Egli, 2010). Many of the factors that alters the absorption of non-haem iron have little effect upon the absorption of haem iron because of the difference in their chemical structure. Iron is released from haem within the intestinal absorptive cell by haem oxygenase and then transferred into the body as non-haem (Wessling-Resnick, 2014).

Some best sources of iron include; beans, lentils, tofu, baked potatoes, cashew, dark green leafy vegetables such as spinach, fortified breakfast cereals, grains and enriched bread etc. (Murray-Kolbe and Beard 2010).

1.3.1 Haem Iron Sources

Haem iron is a biological important iron containing compound and a key source of dietary iron. They are a form of iron found only in blood and muscles tissues, thus 40% is readily absorbed by the blood (Murray-Kolbe and Beard, 2010). Haem iron are gotten from animal foods, however they are readily absorbed and not well regulated by the body. Once ingested and absorbed, the body has no mechanism to remove excess iron (Short and Domagalsk, 2013).

Good sources of haem iron include; beef, pork, chicken, veal, fish such as halibut, haddock, perch, salmon or tuna, shellfish such as clams, oysters and mussels etc.

1.3.2 Non-Haem Iron Sources

The body naturally regulates iron absorption from plant based sources, thus preventing iron overload. It is estimated that 85-90% of total iron intake comes from the non-haem form while 10-15% comes from the haem form (Hurry and Egli, 2010). In terms of bioavailability, non-haem iron is absorbed much less efficiently than haem iron (Hurry and Egli, 2010).

Good sources of non-haem iron include; fortified cereals, rice wheat and oats, dried fruits like raisins and apricots, dark green leafy vegetables like spinach and kale, beans like lentils and soybeans.

Non-haem iron is also found in animal products such as eggs or milk/dairy and it also comprises more than half the iron contained in animal diet. Animal meat is a combination of haem and non-haem, dairy and eggs are non-haem then plant foods are non-haem only (Wessling-Resnick, 2014).

1.4 Iron Deficiency in Pregnant Women

iron deficiency is the most common nutritional deficiency in the world (WHO, 2015). When loss of iron is not adequately compensated by adequate dietary iron intake, a state of latent iron deficiency occurs, which will over time lead to iron deficiency anaemia if left untreated, which is characterized by an insufficient number of red blood cells and an insufficient amount of haemoglobin (Okafor *et al.*, 2013). Iron deficiency is caused by blood loss and this can occur through childbirth, uterine fibroid, stomach ulcers, colon cancer, urinary tract bleeding and heavy period before the woman got pregnant, insufficient dietary intake (especially in adolescent girls) or poor absorption of iron from food (Milman, 2006).
Iron deficiency may also develop during pregnancy even in a well-nourished woman, due to the increase iron requirement of the developing foetus. Iron deficiency can be prevented by eating diet containing sufficient amounts of iron or by iron supplementation (Baird-Gunning *et al.*, 2010).

Iron deficiency anaemia develops in three stages;

1. A depletion of iron stores with no functional impairment. Serum ferritin concentration of less than 12 µg/l support the above statement (WHO, 2015).
2. Deficient erythropoiesis: haemoglobin level remains normal, but red cell protoporphrin is increased. The synthesis of transferrin is increased and percentage saturation is decreased. Then 15% or less saturation is reached, work capacity is impaired (Vasudevan *et al.*, 2011).
3. Iron deficiency anaemia: there is a low haemoglobin resulting in a microcytic hypochromic anaemia. Low stainable iron is seen in born marrow. Only in the late stages of iron deficiency anaemia are low concentration of serum iron observed (Vasudevan *et al.*, 2011)

About 95% of anaemia cases during pregnancy are iron deficiency anaemia. Without iron supplementation, iron deficiency anaemia occurs in many pregnant women because their iron stores need to serve their own increased blood volume, as well as be a source of haemoglobin

for the growing baby and for placental development (Decsi and Lohner, 2014). Children, premenopausal women (women of child bearing age), and people with poor diet are most susceptible to the disease. Most cases of iron deficiency anaemia are mild, but if not treated can cause a problem like fast or irregular heartbeat, complications during pregnancy and delayed growth in infants and children (Scholl, 2005).

1.5 Risk Factor Associated with Iron Deficiency in Pregnant Women

The risk factor of developing iron deficiency during pregnancy increased in women who did not receive iron supplementation during pregnancy when compared to those who receive iron supplementation and this may be due to increased iron requirement to supply the expanding blood volume of the mother and the rapidly growing foetus and placenta (Allen, 2000). All pregnant women are at risk for becoming anaemic, because they need more iron and folic acid (Gibson *et al.,* 2008). The risk is higher when a pregnant woman is vomiting a lot because of morning sickness or have had two pregnancies close together or do not eat enough food that are rich in iron or if the woman had anaemia before pregnancy (Kefiyalew *et al.*, 2014).

Around 35 percent of expectant mothers may be at risk of pregnancy complications such as miscarriage or preterm birth as a result of iron deficiency (Kefiyalew *et al.,* 2014). Premature new born are especially vulnerable to the development of anaemia. In this group, storage iron concentration is lower at birth, red blood cells half-life is shorter and rapid catch up growth contributes to further depletion of iron stores (Allen, 2000). Preterm infants are often submitted to multiply phlebotomy procedures during the time spent in neonatal intensive care unit. Physiologic anaemia of the new born infant is characterized by a haemoglobin nadir occurring in the first eight weeks of life, which might in the presence of other pathological processes lead to a more severe degree of anaemia (Chandra *et al.,* 2012).

Several or untreated iron deficiency during pregnancy can increase the risk of having; a preterm or low birth weight infant, a blood transfusion (if they lose a significant amount of blood during pregnancy); people who routinely donate blood may have an increased risk of iron deficiency anaemia since blood donation can deplete iron stores, postpartum depression, having baby with anaemia, a child with developmental delay, heart problem; iron deficient anaemia can lead to a rapid or irregular heartbeat i.e. the heart must pump blood to compensate for the lack of oxygen carried into the blood when anaemic, this can lead to enlarge heart or heart failure (Chandra *et al.,* 2012).

1.6 Causes of Iron Deficiency Anaemia in Pregnant Women

Iron deficiency anaemia occurs when the body does not have enough iron to produce haemoglobin which is the part of red blood cells that gives blood its red colour and enables the red blood cells carry oxygenated blood throughout the body (WHO, 2011). If enough iron is not consumed and too much iron is lost, the body cannot produce enough haemoglobin, and iron deficiency anaemia will eventually develop (WHO, 2011).

Causes of iron deficiency anaemia include;

1. BLOOD LOSS: Blood contains iron within red blood cells, some iron is being lose when blood is lost in the process. Women with heavy periods are at risk of iron deficiency anaemia because they lose blood during menstruation (Kassebaum, 2016). Slow, chronic blood loss within the body such as from a peptic ulcer, a hiatal hernia, a colon polyp or colorectal cancer can cause iron deficiency anaemia. Gastrointestinal bleeding can result from regular use of some over the counter pain relievers, especially aspirin (Schrier, 2016).
2. LACK OF IRON IN THE DIET: the body regularly gets iron from dietary food, if little iron is being consumed for some time the body can become iron deficient. For proper growth and development, infants and children needs iron from their diet (schrier, 2016).
3. INABILITY TO ABSORB IRON; Iron from food is absorbed into the bloodstream in the small intestine, an intestinal disorder such as celiac disease, which affect the intestine's ability to absorb nutrients from digested food, can lead to iron deficiency anaemia or if part of the small intestine has been by passed or removed surgically, that may affect the ability to absorb iron and other nutrients (Sharp and Srai, 2007).
4. PREGNANCY; Without iron supplementation, iron deficiency anaemia occurs in many pregnant women because their iron stores need to serve their own increased blood volume as well as be a source of haemoglobin for the growing foetus (Sharp and Srai, 2007).

1.7 Symptoms of Iron Deficiency in Pregnant Women

Initially, iron deficiency can be so mild that it goes unnoticed. But as the body becomes more sufficient in iron and anaemia worsens, the signs and symptoms intensify (Short and Domagalski, 2013).

Iron deficiency anaemia signs and symptoms may include; extreme fatigue, weakness, pale skin, chest pain, fast heart beat or shortness of breath, headache, dizziness or light-headedness, inflammation or soreness of the tongue, brittle nails, poor appetite, especially in infants and children with iron deficiency anaemia (Short and Domagalski, 2013).

1.8 Prevalence and Etiology of Anaemia and Iron Deficiency in Pregnant Women

Iron deficiency is the most common and widespread nutritional disorder in the world (Kraff *et al.,* 2012), as well as affecting a large number of children and women in non-industrialized countries, it is the only nutrient deficient which is also significantly prevalent in virtually all industrialized nations (Sharma, 2003). The world prevalence of anaemia in pregnant women is about 51% (WHO, 2005). In Europe and North America, 10% of the women of childbearing age are anaemic (Haemoglobin less than 7.5 mmol/d or 12 g/dl), (WHO, 2015).

During pregnancy there is a significant increase in the amount of iron required to increase the red cell mass, expand the plasma volume and allow the growth of the fatal placenta unit. Women in their reproductive years often have a dietary iron intake that is too low to offset losses from reproduction (Vasudevan *et al.,* 2011). Consequently, the overall prevalence of iron deficiency in non-pregnant women of reproductive age in the united states 9%-11% is higher than at other age apart from infancy. The prevalence of iron deficient anaemia in the same group is 2%-5% (Hogue, 2016). It is estimated that less than 50% of women do not have adequate iron stores for pregnancy (Yip 2011). Because the iron required for pregnancy (3-4 mg/d) is substantial, risk of iron deficiency and iron deficiency anaemia should increase with gestation (Short and Domagalski, 2013).

During pregnancy anaemia increases greater 4-folds from the 1st and 3rd trimester in the low income women monitored as part of pregnancy nutritional surveillance by the centre for disease control and prevention (CDC, 2002). In a study where the cohort was mostly minority, the data

(2000-2004) suggested that the prevalence of anaemia increases 6-folds from 6.7% (1st trimester) and 27.3% (2nd trimester) to 45.6% (3rd trimester) (Lao *et al.*, 2000).

1.9 Dietary Recommendation of Iron

The U.S institute of medicine (IOM) updated Estimated Average Requirement (EARs) and Recommended Dietary Allowances (RDAs) for iron in 2001 (Hoppe *et al.*, 2005). The current EAR for women ages 14-18 years is 7.9 mg/day. 8.1 mg/day for ages 19-50 years. The RDA is 15.0 mg/day for women ages 15-18 years, 18.0 mg/day for 19-50 years, and 8.0 mg/day thereafter. For men 8.0 mg/day for ages 19 and up. The RDAs are higher than average requirements (Hoppe *et al.*, 2005).

The RDA for pregnancy is 27 mg/day and for lactation 9 mg/day (Milman, 2006). For children age 1-3 years 7 mg/day, 10 mg/day for ages 4-8 years and 8 mg/day for ages 9-13 years. As for safety, the U.S institute of medicine also tolerance upper intake levels (ULs) for vitamins and minerals when evidence is sufficient. In the case of iron, the UL is set at 45 mg/day. Collectively the EARs, RDAs and ULs are referred to as Dietary Reference Intake (CDC, 2002).

The European Food Safety Authority (EFSA) refers to the collective set of information as Dietary Reference Valve, with population Reference Intake (PRI) instead of RDA and Average requirement instead of EAR (CDC, 2002). AL and UL defines the same as in united states. For women the PRI is 13 mg/day for ages 15-17 years, 16 mg/day for women ages 18 and up who are premenopausal and 11 mg/day for post-menopausal (CDC, 2002).

For pregnancy and lactation, 16 mg/day. For men the PRLs higher than the U.S RDA with the expectation of pregnancy (Beard, 2000). Frequent blood donors are at risk of low iron levels and are often advised to supplement their iron intake (Beard, 2000).

1.10 Iron Requirement During Pregnancy

If the demand of iron were spread evenly throughout gestation, iron supplement could be met easily by a sustained rise in the rate of iron absorption. The need for iron however varies markedly during each trimester of pregnancy (Beaton, 2000).

The body iron requirement for an average pregnancy is approximately 1,000 mg. It is calculated that 350 mg of iron is lost to the foetus and the placenta and 250 mg is lost in the blood at delivery (Beaton, 2000). In addition, about 450 mg of iron is required for the large increase in maternal red blood cell mass. Lastly, basal loss of iron from the body continue during pregnancy and amount to about 240 mg. thus the total amount iron requirement for a pregnancy (excluding blood loss at delivery) average about 1040 mg. permanent iron loss during pregnancy include loss to the foetus and placenta, blood loss at delivery, and basal losses, which together total 840 mg (Tapiero *et al.,* 2001).

The total iron needs of slightly more than 1,000 mg are concentrated in the last two trimester of pregnancy. This amount is equivalent to about 6mg of iron absorbed per day in a woman who start pregnancy with absent or minimal storage iron (Tapiero *et al.,* 2001). Although 450 mg of iron for red cell production must be supplied during pregnancy, a large part of this can subsequently augment iron stores after a vaginal delivery, when the red blood mass decreases. The result is analogous to spontaneously increase within a few months after delivery in most women who develop mild iron deficiency during late pregnancy because of the iron that is release by the decline in red cell mass (Scholl and Reilly, 2000). Postpartum iron status is also improved by the decreased iron loss during this period: less than 0.3 mg/day is lost in human milk and menstruation is rare in women during their first few months of lactation (Scholl and Reilly, 2000).

The average blood loss during a caesarean delivery is almost twice that occurring with the average vaginal delivery of a single foetus. postpartum improvement in iron status may therefore be less complete after a caesarean delivery (Kassebaum, 2016).

1.11 Iron Supplementation

Iron supplementation during pregnancy has not been clearly showed to affect pregnancy output, it improves maternal iron status parameters. The effectiveness depends upon the dosage and the form in which it is administered (National Institute of Health, 2015). In developed countries most women enter pregnancy with normal haemoglobin concentration and variable amount of stored iron. Large amount of women is anaemic at the onset of pregnancy (National Institute of Health, 2015).

In 2015 the World Health Organization (WHO) recommended universal supplementation of all pregnant women with 60 mg ferrous iron twice daily in population where gestational

anaemia is common and once daily in population where overall nutrition is better (Ronsmans and Graham, 2006). Iron requirement during pregnancy is between 800 mg and 1000 mg depending on the size of the women (45-55 kg). iron absorption from a diet of a very high iron bioavailability has been estimated to be 0.4, 1.9 and 5.0 mg/d during the first, second and third trimester respectively. A diet with the above absorption rates would provide a total of 600 mg Fe during pregnancy, leaving a deficit of 200-400 mg Fe that would have to be met by mobilizing iron from stores, if they exist and from the absorption of supplemental iron (Bothwell, 2000). A daily dose of 30 mg ferrous iron during the daily absorption rate that would appear to be adequate because the daily absorption rate that would be required to make up the deficit would be at most approximately 10% (Bothwell, 2000).

The problem of anaemia during Pregnancy is compounded by the fact that many women consume diet of low bioavailability and, therefore enter pregnancy with no iron stores and less than optimal haemoglobin concentration (Ronsmans and Graham, 2006). Between 200 mg to 400 mg, iron can be absorbed from diet with low to medium bioavailability; thus a deficit of as much as 600 mg - 800 mg must be met from supplementation (Picciano, 2003). The extra amount of iron that would have been absorbed to meet such deficit would be 5.5 - 7 mg/d, if supplementation was started at 20 weeks of gestation and double the concentration of it were started at 30 weeks (Beard, 2008).

These amount would be meet if 9 - 12% and 19 - 24% of the 60 mg ferrous iron tablet given in the fastening state at 20-30 weeks of gestation respectively is absorbed. It is apparent that a daily dose of 60 mg ferrous iron given to fasting pregnant women throughout the second half of pregnancy should be sufficient to combat iron deficiency in developing countries (Beard, 2008). A dose of 120 mg/d should be required only when supplementation therapy is not started at the beginning of the second trimester (Bothwell, 2000).

1.11.1 Limitation of Iron Requirement

Programmatically, several factors can limit the effectiveness of iron supplement interventions, including problems related to cost and logistics that effect the supply of iron tablets, poor access to prenatal care, insufficient counselling on the need for and benefit of iron supplementation (Picciano, 2003). Available literature from several countries suggest that the most important reason for the failure of supplementation programs is a lack of supplies, but noncompliance on the part of pregnant women can also be a significant factor (Lieu *et al.*, 2001).

Noncompliance is the result of both an aversion to the side effect of taking iron supplement and the failure of many primary health care systems to adequately motivate both health care providers to issue the iron tablets and pregnant women to take them (Bothwell, 2000). Various strategies have been adopted to reduce the gastrointestinal side effect associated with taking iron supplement such as nausea and epigastric pain, which are important factors in noncompliance. Side effect are dose related; thus a reduction in both the concentration and frequency of the oral iron dose has been advocated (Lieu *et al.*, 2001).

The minimum effective dose of iron for a woman entering pregnancy with no iron stores is 60mg/d and may even be greater for those who are already anaemic at the onset of pregnancy. An alteration approach is to administer the iron in a form that is well absorbed and likely to produce fewer side effect (Yip, 2001). A formulation referred to as a gastric delivery system (GDS) which consist of ferrous sulphate incorporation into a hydrocolloid matrix that becomes buoyant on exposure to gastric secretions; thus, the gastric delivery is retained for a prolonged period in a soluble form in the acidic environment of the stomach (Yip, 2001).

1.12 Iron Metabolism

After birth, excluding exogenous therapeutic sources, all iron enters the body from diet. Dietary iron is usually considered as either haem or non-haem. Non-haem is abundant in foods of both animal and plant origins and is the dominant form of iron in plants. Non-haem iron is found in a wide variety of forms and includes soluble iron, iron in low-molecular-weight complexes, storage iron in ferritin, and iron in the catalytic centres of a wide range of proteins (Hentze *et el.*, 2004).

Many of this iron is not tightly sequestered, and consequently its bioavailability can be affected by a range of dietary constituents and luminal factors. The low pH of the stomach and proximal small intestine helps to keep iron in a soluble form, thereby making it available for absorption. Small organic acids such as citric acid and ascorbic acid also help to keep non-haem iron in a reduced and soluble form and can greatly enhance its absorption (Hentze *et al.*, 2004).

Other dietary components, notably plant-derived phytates, tannins and polyphenols, can bind non-haem iron and impede its absorption. In contrast, haem iron is tightly sequestered within a protoporphyrin ring and is not accessible to the factors that influence non-haem iron. As a consequence, haem iron tends to be absorbed more efficiently and its absorption is less

dependent on the composition of the diet. Most haem iron in the diet is from myoglobin and haemoglobin and is animal derived (McKie *et al.*, 2001).

1.12.1 Intestinal Iron Absorption

Iron is absorbed by the mature enterocytes of the mid-upper villus and mainly in the small intestine. Although small amounts of iron can be absorbed by the more distal parts of the gastrointestinal tract, the proximal parts of the small intestine (the duodenum and the first part of the jejenum) are particularly adapted for this role (McKie *et al.*, 2001).

Non-haem iron enters the circulation after traversing the enterocyte apical membrane via divalent metal-iron transporter 1 (DMT1) and the basolateral membrane through ferroportin 1 (FPN1). Iron binds to plasma transferrin (TF) and is distributed to tissues throughout the body. Most iron is used by immature red blood cells in the bone marrow for haemoglobin production. Senescent erythrocytes are phagocytosed by macrophages, and the iron is released from catabolized haemoglobin and re-enters the circulation (McKie *et al.*, 2001).

The DMT1 requires ferrous iron as a substrate, but most dietary iron is in the ferric form. Thus iron needs to be reduced before it can be absorbed. Duodenal cytochrome B is one potential brush-border reductase (McKie *et al.*, 2001).

Haem iron is presumed to bind to the enterocyte brush border intact and, then is likely to be endocytosed but the molecular details are not known. Once in the enterocyte, it is considered that iron is released from haem through the action of haem oxygenase and that this iron is subsequently exported from the cells via FPN1 in the same way as non-haem (Theil *et al.*, 2012).

1.12.2 Systemic Iron Delivery to Tissues

The recently absorbed iron or iron released from storage site is bound to plasma transferrin which distributes it around the body to sites of utilization (Frazer and Anderson, 2014). Each transferrin molecule can bind two or more atoms of iron. Under normal circumstances, only 30% of the iron-binding sites on the plasma transferrin pool are occupied at any one time (that is, a transferrin saturation (TSAT) of 30%), thereby providing considerable buffering capacity against the appearance of potential toxic non-transferrin-bound iron (NTBI). However, in iron-loading diseases, transferrin frequently becomes saturated, and the concentration of NTBI can be dangerously high (Fleming and Ponka, 2012). TSAT can provide a useful index of iron

supply to the marrow, and TSAT less than 16% is correlated with a reduced production of new red blood cells (Ohgami *et al.*, 2005).

Diferric transferrin delivers iron to cells by binding to transferrin receptor (TfR), on the plasma membrane (Frazer and Anderson, 2014). The transferrin-TfR 1 complex is internalized via clathrin-mediated endocytosis. The endosome is acidified, and a combination of low pH, a conformational change in transferrin that is associated with its binding to its receptor, and a reduction of the transferrin-bound ferric iron via an enzyme of the 6-transmembrane epithelial antigen of the prostate family of reductases (6-transmembrane epithelial antigen of the prostate 3 in the case of immature erythroid cells) releases iron from transferrin (Park *et al.*, 2001).

This iron moves into the cytoplasm across the endosomal membrane via DMT 1 (Park *et al.*, 2001). The fate of this iron depends on the iron requirements of the cell. If iron is needed for metabolic functions, it may move directly to sites of utilization such as the mitochondria. If iron is not immediately required, it may be sequestered within the iron storage protein ferritin for later use (Donovan *et al.*, 2005).

1.12.3 Intracellular Iron Trafficking and Storage

Iron storage is a critical aspect of cellular iron homeostasis, which enables iron to be sequestered in a nontoxic form, and also provides a reservoir from which iron can be used for future metabolic needs. Ferritin is the main intracellular iron storage protein (Theil, 2013).

Ferritin is a large protein that consist of 24 subunits that are arranged to form a spherical shell with a large central cavity. Pores in the protein shall enable the entry and exit of iron, and a single ferritin molecule can hold 4,500 or more atoms of iron. This high capacity, combined with the fact that the expression of ferritin greatly increases when cellular iron concentration rise, provides the cell with an enormous ability to sequester iron (Theil, 2013).

When high concentrations of iron-laden ferritin accumulate within the cell, the ferritin molecules aggregate, and ultimately, these aggregates fuse with lysosomes. This process leads to the degradation of ferritin, and resulting mixture of ferric iron cores and peptides is known as hemosiderin (Theil, 2013).

This form of storage iron is particularly prominent in the cells of patients with iron-loading diseases. Iron can be efficiently mobilized from both ferritin and hemosiderin when it is required elsewhere in the body (Theil, 2013).

1.12.4 Regulation of Cellular Iron Homeostasis

Cellular iron homeostasis is tightly regulated to maximize the iron supply when the cell has an iron deficit and to restrict the iron supply and promote storage when the cell is iron replete. This regulation can occur at different levels. At the molecular level, when cellular iron concentrations are low, the iron regulatory proteins (IRPs) are in their mRNA binding conformations. The binding of IRPs to the 5'UTR of the ferritin mRNA blocks translation, thus ensuring that little ferritin is produced when iron storage is not required (Wilkinson and Pantopoulos, 2014).

Concurrently, the IRPs bind to iron-responsive elements (IREs) in the 3'UTR of the TFR1 m-RNA, thereby protecting the message from degradation and enabling more TfR1 to be expressed on the cell membrane. This effect, in turn, enables the cell to maximize its iron intake. When cellular iron concentrations are high, the IRPs do not bind to IREs, thereby allowing translation of the ferritin m-RNA to proceed and exposing the TfR1 m-RNA to degradation. These conditions limit cellular iron uptake and promote storage (Wilkinson and Pantopoulos, 2014).

The regulation of iron homeostasis at the cellular level is replicated at the whole-body level. If an individual requires more iron, more iron will be mobilized from body iron stores and there will be an increase in intestinal iron absorption. However, if the body is iron replete, these processes will be downregulated. This regulation is mediated by the small liver-derived peptide hepcidin (Ganz, 2013).

Variations in body iron demand are communicated to the liver and this, in turn, modulates the expression of hepcidin, which is encoded by the hepcidin antimicrobial peptide gene. Hepcidin is distributed via the circulation to its target sites where it binds to the iron export protein FPN1. Thus, FPN1 acts as the hepcidin receptor. When the body iron is replete, hepcidin concentrations are high, and the iron supply to the plasma is reduced; however, when iron demands are high, hepcidin concentrations are reduced, and more iron enters the circulation (Ganz, 2013).

1.12.5 Organ-Specific Iron Metabolism

Different organs have varying iron requirements, even within individual tissues, different cell types differ in their capacity to take up various forms of iron and vary in their capacity to store iron. The erythroid marrow has the highest iron requirement of any body tissue because a very

large amount of iron is required for haemoglobin synthesis in new red blood cells (Fleming *et al.*, 2012).

Tissues with a high proliferative capacity such as rapidly dividing cells of the intestinal crypts also require relatively large amounts of iron and, situation is also seen in pregnancy when the iron supply to the foetus is prioritized at the expense of the mother (Milman, 2006).

1.13 Biomarkers of Iron Deficiency Anaemia

Some of the haematological parameters include the following; haemoglobin, total serum iron, ferritin, red blood cell and malaria parasite.

1.13.1 Haemoglobin

Haemoglobin is a protein molecule in red blood cells that carrier's oxygen from the lungs to the body's tissue and returns carbondioxide from the tissue back to the lungs (Voet, 2008). They are made up of four protein molecules (globulin chains) that are connected together. The normal adult haemoglobin molecule contains two alpha-globulin and two beta globulin chains. In foetus and infants, beta chains are not common and the haemoglobin is made up of two alpha chains and two gamma chains. As the infant grows, the gamma chains are gradually replaced by beta chains, forming the adult haemoglobin structure (Nelson and Cox, 2000). Each globulin contains an important iron-containing porphyrin compound termed haem and within the haem compound is an iron atom that is vital in transporting oxygen and carbondioxide in the blood. The iron contained in haemoglobin is also responsible for the colour of blood (Nelson and Cox, 2000).

A low haemoglobin level is referred to as anaemia or low red blood cell count. Dehydration also produces a falsely high haemoglobin measurement that disappears when proper fluid balance is restored (Voet, 2008).

Some other infrequent causes of high haemoglobin level include; advance lungs disease (for example emphysema), certain tumours, a disorder of the bone marrow in which it makes too much red blood cell, known as polycythaemia, abuse of the drug erythropoietin (Epogen) by athletes for blood doping purposes (increasing the amount of oxygen available to the body by chemically raising the production of red blood cells) (Saha and Reddy, 2014).

Low haemoglobin level that is needed to be increased are caused by three circumstances; decreased red blood cell production (for example bone marrow haemoglobin production, iron deficiency), increased red blood cell destruction (for example, liver disease), and by blood loss (for example, trauma form a gunshot or knife wound) (Saha and Reddy, 2004).

1.13.1.1 Ways of Increasing Haemoglobin Level

Ways of increasing haemoglobin level are varied and their use depends on the underlying problems (Murray-Kolbe and Beard, 2010).

Some of the ways to increase haemoglobin include; transfusing red blood cells, receiving erythropoietin – a hormone used to stimulate red blood cell production in individuals with decreased red blood cell production or increased red cell destruction, taking iron supplement, increasing the intake of iron-rich foods (eggs, spinach, beans, lean meat and sea food) and food rich in cofactors such as (vitamin B6, folic acid, vitamin B12, and vitamin c) important for maintaining normal haemoglobin level (Murray-Kolbe and Beard, 2010).

1.13.2 Red Blood Cell Count (RBC)

Red blood cells also referred to as red cells, red blood corpuscles and erythrocytes etc. are the most common type of red blood cell and the vertebrate's principal means of delivering oxygen (O_2) to the body tissue via blood flow through circulating system (Jeremy *et al.*, 2012). The cytoplasm of erythrocytes is reach in haemoglobin, an iron containing biomolecules that can bind oxygen and is responsible for the red colour of the cells and the blood (Jeremy *et al.*, 2012).

As a result of not containing mitochondria, red blood cells used none of the oxygen they transport; instead they produce the energy carrier ATP by glycolysis of glucose and lactic acid fermentation on the resulting pyruvate (Tilton *et al.*, 2012). As red blood cells contain no nucleus, protein biosynthesis is currently assumed to be absent in these cells. Because of lack of nuclei and organelles, mature red blood cells do not contain DNA and cannot synthesize any RNA and consequently cannot divide and have limited repair capabilities (Tilton *et al.*, 2012). The inability to carry out protein synthesis means that no virus can evolve to target mammalian red blood cells (Jiang *et al.*, 2007).

The human red blood cells are produced through a process named erythropoiesis, developing from committed stem cells to mature red blood cells in about 7 days. Through this process red blood cells are continually produced in the bone marrow of large bones (Kabanove, *et al.*, 2009). In the embryo, (the liver is the main site red blood cell production) the production can be stimulated by the hormone erythropoietin (Epo), synthesized by the kidney just before and after leaking the bone marrow, the developing cells are known as reticulocytes. This constitute about 7% of circulating red blood cells (Kabanove *et al.*, 2009).

1.13.3 Serum Ferritin

Ferritin is a universal intracellular protein that stores iron and releases it in a controlled way. In humans, it acts as a buffer against iron deficiency and overloading (Wang *et al.*, 2010). It is found in most tissues as a cytosolic protein, but small amount is secreted into the serum where it functions is an iron carrier. Ferritin that is not combined with iron is called apoferritin (Torti and Torti, 2002).

If the ferritin level is low, there is a risk of lack of iron, which could lead to anaemia. In the settings of anaemia, low serum ferritin is the most specific laboratory test for iron deficiency (Hogue, 2006). However, it is less sensitive, since its levels are increased in the blood by infection or any type of chronic inflammation and this condition may convert what could otherwise be a low level of ferritin from lack of iron (Hogue, 2006). Low level of ferritin may also indicate hypothyroidism, vitamin c deficiency or celiac (Torti and Torti, 2002).

If ferritin level is high, there is iron in excess or else there is infection without signalling body overload (Orino *et al.*, 2001). Ferritin is also used as bench marker for iron overload disorders such as hemochromatosis or hemosiderosis. Adult-onset still disease, some haemophagocytic lymphohistocytosis/macrophage activation syndrome are diseases in which ferritin level may abnormally raise (Goodman *et al.*, 2007)

1.13.4 Malaria

Iron deficiency is very common in malaria endemic areas. It causes anaemia and in young children iron deficiency is associated with neurodevelopmental delay (Breman *et al.*, 2001). Pregnant women are particularly vulnerable to malaria as pregnancy reduces a woman's immunity to malaria, making them more susceptible to malaria infection and increasing the risk of illness, severe anaemia or death. For the unborn child, maternal malaria increases the

risk of spontaneous abortion, stillbirth, premature delivery and low birth weight; a leading cause of child mortality (Breman *et al.*, 2001). Malaria anaemia is thought to arise from both decrease red blood cell (RBC) production and increased RBC destruction. Destruction of red blood cell can occur as a result of parasite invasion and replication (Waitumbi *et al.*, 2000).

Malaria is associated with increased acute phase protein concentration and severe malaria increases erythrocyte rigidity, which may affect the relationship between haemoglobin and haematocrit (Urban and Roberts, 2002). An infected female anopheles' mosquito inoculates sporozoites into the human host (Waitumbi *et al.*, 2000). Sporozoites are cells that develop in the mosquito glands, leave the mosquito during blood meal, and enter the liver cells (hepatocytes), where they multiply, cells infected with sporozoites eventually burst, releasing non – motile merozoites into the blood stream and they infect the red blood cells (Price *et al.*, 2001). Both *Plasmodium falciparum* and *Plasmodium vivax* induce anaemia, and severe anaemia caused by *P. falciparum* is responsible for approximately a third of the death associated with diseases (Price *et al.*, 2001).

Malaria infection are the major global causes of anaemia and measures taken to decrease malaria at a population level also decrease anaemia (Abrams and Meshnick, 2009).

1.14: Rationale for the Study

There is a current concern that irrespective of worldwide campaign against anaemia in pregnancy, women of reproductive age still enter into pregnancy without proper iron stores and could give birth to children with inadequate iron deposit.

1.15: Aim and Objective of Study

This study was aimed at determining the iron status of pregnant women in Nsukka, Enugu State

To reach this aim, the specific objectives of the study are as follows;

1. To determine the concentration of haemoglobin in pregnant women
2. To determine the concentration of serum ferritin in the subject**s.**
3. To use combined ferritin and haemoglobin concentration to determine the prevalence of anaemia in the subjects.
4. To determine the effect of age, gestational age (trimester) and intake of routine drugs on haemoglobin and ferritin concentration of the subjects.

CHAPTER TWO

MATERIALS AND METHODS

2.1 MATERIALS

2.1.1 Subjects

One hundred and twenty-seven pregnant women at different trimester (6 were in the first trimester; 50 were in the second trimester and 71 were in the third trimester), all living at Nsukka community, Enugu State, Nigeria, were recruited for this study. Ethical approval was obtained from Nsukka Divisional Health Centre and the Ethical Committee of Faculty of Biological Sciences. The objectives and benefit of this study was carefully explained to the subject before they accept to take part in the study.

2.1.2 Study Area

Pregnant women attending the antenatal clinic in Nsukka Divisional Health Centre were recruited for the study. This is the major public maternity unit within Nsukka community. The hospital gives quality healthy service and sensitize pregnant women on how to take care of themselves during the course of their pregnancy.

2.1.3 Equipments and Instruments

Equipment and instrument used for the study were obtained from Shalom Research Laboratory and Projex Laboratory and other scientific shop in Nsukka. They include; centrifuge (model 800D; New life medical instrument, England), spectrophotometer (model SPM721- 2000, Biodiagnosis Inc., USA), refrigerator (Haier thermocool, England), non-anticoagulated bottles, EDTA bottles, micropipette (Volume Range 0-1000µl; Swastic Scientific Instrument Private Ltd, Mumbai India), microtiter plate reader (model MR 9602A; Biotech USA).

2.1.4 Chemicals/Reagent

Analytical grade chemicals were used in this study. The reagents and chemicals used for haemoglobin determination were Darbkin's reagent which contain potassium cyanide, potassium ferricyanide, and sodium bicarbonate. Calbiotech kits determination ferritin which contain biotin reagent, streptavidin coated microwells, ferritin reagent (horseradish peroxide),

ferritin calibrator, substrate A (tetramethybenzidine), substrate B (hydrogen peroxide) and stop solution (hydrochloric acid).

2.2 METHODS

2.2.1 Data Collection

The following were the inclusive criteria for selection of subject; their age, trimester, routine drug, pregnancy history, resident within Nsukka community, Enugu State, consent to participate in the study, willingness to give 5 ml of blood.

2.2.2 Sample Collection

A known volume, 5 ml of blood sample was collected from each participant by venepuncture using disposable needles and syringes. Thereafter 3 ml of the blood sample were transferred into a labelled plane container. The blood was allowed to clot, spun at the speed of 4000 rmp for 10 minutes using centrifuge. The serum obtained was carefully separated using Pasteur pipette and transferred to a new plain container, while the whole blood was discarded properly according to the best practice of laboratory waste disposal. The remaining 2 ml of the blood sample was transferred into ethylene diaminetetracetic acid (EDTA bottle). It was properly kept for malaria parasite and haemoglobin determination.

2.2.3 HAEMOGLOBIN DETERMINATION

Haemoglobin determination was done using standard method as described by Ochei and Kolhalther (2008).

Principle

The haemoglobin determination is based on light intensity principle on the dilution of blood (from EDTA) in a solution called Drabkin's solution, which contains potassium cyanide and potassium ferricyanide. Red blood cells are haemolysed and haemoglobin is released which is converted to methaemoglobin by reacting with potassium ferricyanide. The methaemoglobin is converted to cyanmethaemoglobin (which has maximum absorbance of 540nm) by potassium cyanide. The colour intensity measured at 540 nm is proportional to the total haemoglobin concentration.

Procedure

The blood sample (20 µl) was diluted in 8 ml of Drakin's solution by 1:250. The tube was then covered and inverted several times and left to stand for 5 minutes to ensure complete conversion. The cyanmethaemoglobin (HiCN) was poured into a cuvette. The spectrophotometer was set to 100% transmitter at 540 nm using Drabkin's solution as blank. The haemoglobin value was then determined by multiplying by a factor of 36.8 to give the actual haemoglobin value.

2.2.4 FERRITIN DETERMINATION

Serum ferritin determination was done using enzyme immunoassay method as described by Addison (1972) and as contained in Calibiotech ferritin kit.

Principle

The principle is based on enzyme reaction where streptavidin coated micro well react with ferritin biotin reagent (monoclonal biotinylated antibody) and the serum containing the native antigen from an antibody-antigen complex. The biotin attached to the antibody binds to streptavidin on the micro well resulting in immobilization of the complex. After incubation the antibody-antigen bound fraction is separated from unbound antigen by decanting. Another antibody that is directed to a different epitome labelled with an enzyme was added leading to an enzyme labelled antibody-antigen-biotinylated complex. Excess enzyme is washed out using the washing buffer. A suitable substrate is added to produce a colour effect measurable with the use of spectrophotometer.

Procedure

Prior to assay, the reagent was allowed to stand at room temperature (20 - 25^0c). The reagent was gently mixed before use.

The desired number of coated strips was placed in to the holder for patient's specimen to be assayed in duplicate.

A known volume, 25 µl (0.02 ml) of ferritin standard, control and samples was Pipette into appropriate wells and 100 µl of biotin reagent was added into each well. Thereafter the plate was shaken for 10 – 30 minutes. The plate was covered and incubated for 30 minutes at room

temperature (20 - 25^{0}c). Liquid was removed from all wells and washed well for three times with 30x wash buffer. The plate was tapped and blotted dry with absorbance paper. After which 100 µl (0.100 ml) of the enzyme reagent was added into each well and was covered and incubated for 30 minutes at room temperature (20 - 25^{0}c). The liquids from all wells were removed and washed for three times with 300x wash buffer. The plate was tapped and blotted dry using absorbance paper. The substrate, 100 µl (0.100 ml) was added into all wells and incubated for 15 minutes at room temperature. Thereafter 50 µl (0.050 ml) of stop solution was added into all well and was carefully mixed for 10 -20 minutes at room temperature. The absorbance in each well was read at 450 nm within 15 minutes after adding the stop solution (using wavelength of 620 – 630 nm) in a micro plate reader.

2.2.5 Statistical Analysis

The data was expressed as mean ± standard error of mean (SEM) and test of statistical significance was carried out using one-way analysis of variance (ANOVA). Pearson test was performed to assess the correlation between Haemoglobin concentration, serum ferritin with other variables.

CHAPTER THREE

RESULTS

3.1: Demographical Information on Pregnant Women.

Table 1 shows the demographical information of pregnant women. One hundred and twenty-seven pregnant women aged 20 – 49 years participated in the study. The age distributions were 20 – 24 years (26.8%), 25 – 29 years (37%), 30 – 34 years (20.4%), 35 – 39 years (12.6%), 40 -44 years (2.4%) and 45 – 49 years (0.8%). Among the pregnant women used in this study, six (4.7%) were in their first trimester, fifty (39.4%) were in their second trimester and seventy-one (55.9%) in their third trimester. A total number of fifty-six (44.1%) took the routine drugs regularly, while seventy-one (55.9%) did not.

3.2 Prevalence of Anaemia Using Haemoglobin Concentration Alone

Table 2 shows the result of prevalence of anaemia using haemoglobin concentration alone. From the table 39.4% of the pregnant women were anaemic based on haemoglobin less than 11g/dl. In first trimester, 0.79% were anaemic, second trimester 18.89% were anaemic while 19.69% were anaemic in the third trimester.

3.3: Prevalence of Anaemia Using Ferritin Concentration Alone

Table 3 shows the prevalence of anaemia using ferritin concentration alone. From the result 27.6% of the subjects were anaemic based on ferritin concentration less than 15 µl. In first trimester 3.94% were anaemic, 11.81% were anaemic in second trimester while 11.81% were anaemic in third trimester.

Table 1: Demographical Information on Pregnant Woman

Participant(n=127)	Number	Percentage (%)
Age (years)		
20-24	34	26.8
25-29	47	37
30-34	26	20.4
35-39	16	12.6
40-44	3	2.4
45-49	1	0.8
Stage of Pregnancy		
1st Trimester	6	4.7
2nd Trimester	50	39.4
3rd Trimester	71	55.9
Intake of Iron Supplements		
Regular	56	44.1
Irregular	71	55.9

Table 2: Prevalence of Anaemia using Haemoglobin Concentration alone (Anaemia =Hb <11 g/dl).

Gestation age (n=127)	Number	Percentage (%)
First trimester	1	0.79
Second trimester	24	18.89
Third trimester	25	19.69
Total	50	39.40

Table 3: Prevalence of Anaemia using Ferritin Concentration alone (Anaemia = ferritin<15 µl).

Gestation age (n=127)	number	percentage (%)
First Trimester	5	3.94
Second Trimester	15	11.81
Third Trimester	15	11.81
Total	35	27.60

3.4: Prevalence of Anaemia using Combine Haemoglobin and Ferritin Concentration in pregnant women in first, second and third trimester.

Table 4 shows the prevalence of anaemia and non-anaemic pregnant women both in first, second and third trimester. From the result none of the subjects were anaemic in first trimester, 26% of the subjects were anaemic in second trimester while 11.3% of the subjects were anaemic in third trimester. Based on ferritin level alone 66.6% of the subjects were anaemic in first trimester, 46% in second trimester while 53.5% of the subjects in third trimester were anaemic. Conversely, 16.7% of the subjects in first trimester, 22% in second trimester while 23.9% in third trimester were anaemic based on low haemoglobin and ferritin level respectively.

3.5: Effect of Age on Haemoglobin and Ferritin Concentration

Table 5 shows the effect on haemoglobin and ferritin concentrations. From the table, mean haemoglobin concentration has a significant different ($p < 0.05$) when age range of 40 – 45 years is compared to other age range. However, the age range of 30 – 34 years are non-significantly ($p > 0.05$) when compared to age range of 35 -39 years and 40 – 44 years. Mean ferritin concentration shows that the age range of 40 – 44 years and 45 – 49 years are significantly ($p < 0.05$) different when compare to the age range of 20 – 24 years, 25 – 29 years, 30 – 34 years and 35 – 39 years. However, the age range of 20 – 25 years and 35 – 39 years are significantly ($p < 0.05$) lower than the age range of 30 – 34 years.

3.6: The Effect of Gestation Age (first, second and third trimester) on Haemoglobin and Ferritin Concentrations

Table 6 shows the of effect gestational age on haemoglobin and ferritin concentrations. from the results, the mean concentration of haemoglobin in the first trimester (11.60 ± 1.86) shows no significant ($p > 0.05$) difference when compared to second (11.03 ± 1.56) and third trimester (11.06 ± 1.64). The mean value of ferritin concentration in the first trimester (16.24 ± 3.10) was significantly ($p < 0.05$) lower when compare to the mean ferritin concentration in the second trimester (75.72 ± 9.50) and third trimester (66.53 ± 5.09).

Table 4: Prevalence of Anaemia using Combine Haemoglobin and Ferritin Concentrations in first, second and third trimester.

Parameters	Prevalence (%)		
	First Trimester (n=6)	Second Trimester (n=50)	Third Trimester (n=71)
Normal Hb and Normal Ferritin	1(16.7)	3(6)	8(11.3)
Low Hb and Normal Ferritin	(0)	13(26)	8(11.3)
Low Ferritin and Normal Hb	4(66.6)	23(46)	38(53.5)
Low Hb and Low Ferritin	1(16.7)	11(22)	17(23.9)

Table 5: The Effect of Age on Haemoglobin and Ferritin Concentrations

Age Range	Mean Hb (g/dl)	Mean Ferritin (µg/l)
20-24 years (n=34)	11.57 ± 1.37	24.87 ± 5.72
25-29 years (n=47)	11.20 ± 1.91	86.84 ± 11.01
30-34 years (n=26)	10.69 ± 1.03	90.06 ± 12.20
35-39 years (n=16)	10.58 ± 1.75	80.99 ± 10.41
40-44 years (n=3)	10.38 ± 0.58	8.32 ± 1.36
45-49 years (n=1)	8.64 ± -	8.78 ± -

Result are expressed in mean ± standard deviation.

Hb = haemoglobin

Table 6: The Effect of Gestation Age (first, second and third trimester) on Haemoglobin and Ferritin Concentrations.

Gestation age	Mean Hb (g/dl)	Mean Ferritin (µg/l)
First Trimester (n=6)	11.60 ± 1.86	16.24 ± 3.10
Second Trimester (n=49)	11.03 ± 1.56	75.72 ± 9.50
Third Trimester (n=72)	11.06 ± 1.64	66.53 ± 5.09

Result are expressed in mean ± standard deviation.

Hb = haemoglobin

3.7: The Effect of Routine Drugs on Haemoglobin and Ferritin Concentrations

From the result, the mean haemoglobin concentration of those that received routine drug (11.30 ± 1.73 g/dl) was slightly higher compared to those who didn't receive the drug (10.59 ± 1.49 g/dl). However, the mean ferritin concentration of pregnant women who receive routine drug (58.53 ± 4.08 µg/l) was significant higher ($p < 0.05$) when compared to the concentration of those that didn't receive (75.17 ± 9.10 µg/l).

3.8: Correlation Analysis of age range, Gestation age and Intake of Routine Drugs on the Haemoglobin and Ferritin Concentrations of Pregnant Women.

The correlation table below, shows that age is negatively correlated with haemoglobin but positively correlated with ferritin. This means that as age of the subjects was increasing, their haemoglobin level was decreasing. This decrease was statistically significant ($p < 0.05$). however, the ferritin level was increasing with an increase in the age of the subjects, although not significant ($p > 0.05$). Gestational age when correlated with the haemoglobin and ferritin concentration shows a negative correlation ($p > 0.05$). This implies that increase as gestational age of the subjects' increases, there was a decrease in haemoglobin and ferritin concentrations. Also, from the result, intake of routine drugs when correlated with the haemoglobin concentration shows a negative correlation ($p > 0.05$) but when correlated with ferritin concentration shows a positive correlation ($p > 0.05$), which means that intake of routine drug seems to have reduce the haemoglobin level but increase ferritin level of the subjects.

Table 7: The Effect of Routine Drug on Haemoglobin and Ferritin Concentrations

Groups	Mean Hb (g/dl)	Mean Ferritin (µg/l)
Regular (n=57)	11.30 ± 1.73	58.53 ± 4.08
Irregular (n=70)	10.59 ± 1.49	75.17 ± 9.10

Result are expressed in mean ± standard deviation.

Hb = haemoglobin

Table 8: Correlation Analysis of Age range, Gestation age and Routine Drugs on the Haemoglobin and Ferritin Concentrations of Pregnant Women.

Groups	Mean Hb (g/dl)	Mean Ferritin (µg/l)
Age	- 0.260**	0.083
Stage of Pregnancy	- 0.36	- 0.021
Intake of Supplements	- 0.126	0.057

Correlation is significant at p- value ($p < 0.05$)

* Correlation is significant at the 0.01 level

** Correlation is significant at the 0.05 level

CHAPTER FOUR

DISCUSSION

Iron deficiency is one of the most important nutritional disorder among pregnant women worldwide. It could directly or indirectly contribute to the high rate maternal, perinatal morbidity and mortality seen in Nigeria (Rodger *et al.,* 2015).

Iron deficiency anaemia was highly prevalent among pregnant women used in this study. Using the World Health Organisation criterion of haemoglobin level below 11 g/dl to define anaemia in pregnant women, 39.4% of the pregnant women used in this study were anaemic, with a total number of 0.79% in first trimester, 18.89% in second trimester and 19.69% in third trimester. This result is in support with the data obtained from previous studies in developing countries showing a range from 35.5% to 75.0% (Omigbodum, 2004). The prevalence of anaemia in this study based on haemoglobin level (39.4%) is within the range with the findings of other studies carried out in Nigeria, Mcmahon, 2013 (40.2%); Anorlu *et al.* 2006 (35.3%); and Barker and Greer, 2010 (51.8%). The 39.4% from this study is however lower than 64.1% reported by Ezugwu *et al.* (2013) in Enugu state. This could be as a result of educational and economic status of the subjects. However, the prevalence of 39.4% is higher than the 10.4% obtained at Ibadan in south-West Nigeria (Pasricha *et al.,* 2010) and 21.6% obtained by Alem *et al.* (2013) in Northwest Ethiopia. This may be due to the differences in feeding habits, routine drug intake or some other factors not considered in their research.

The prevalence of anaemia using ferritin concentration alone from this study shows that 27.6% of the subjects were anaemic. This is based on ferritin concentration below 15 µg/l, with 3.94% in first trimester, 11.8% in second trimester and 11.8% in third trimester. Late antenatal booking may have contributed to a rise in prevalence, since most of the subjects in this study started their antenatal booking during the second and third trimester (Owolabi and Olalorum, 2014).

The result of haemoglobin on gestational age (Trimester) from this study shows higher value (11.60±1.86) in first trimester when compared to second (11.03±1.56) and third (11.06±1.64) trimester. This result is in accordance with Kumar *et al.* (2013) and Goswami *et al.* (2014), which reported that increase in gestational age does not affect haemoglobin concentration. Also Hogue (2016) reported that the prevalence of anaemia among pregnant women of in Bida, Niger State based on haemoglobin level shows no difference when compared with other

trimester. This could be as a result of greater expansion of plasma volume with increase in red blood cell volume (Perez *et al.*, 2005). Plasma volume expands nearly 50% and consequently haemoglobin level decreases. However, ferritin level in second trimester was higher when compared to first and third trimester. According to Bencarova and Breymann (2014), serum ferritin concentration is maximum in second trimester and then falls with advance gestational age in third trimester. This could be as a result of iron supplement consumption by the subject in second trimester since they normally start their prenatal care in second trimester. By third trimester, the iron demand by the foetus increases leading to a general decline in ferritin levels. Gestational age when correlated with haemoglobin and ferritin concentration shows a negative correlation ($p > 0.05$). This implies that as gestational age of the subject increases, there was a decrease in maternal haemoglobin and ferritin concentration. This result agrees with the report of Demmouche *et al.* (2011) and Nik *et al.* (2012).

The result of the effect of age on this study shows a slightly levels of haemoglobin concentration among subjects within the age of 20 – 24 years and 25 – 29 years but lower levels among subjects within the age of 30 -34 years and 45 – 49 years. This is to say that haemoglobin level decreases as age increases. This could be as a result of the physiological function of the cells (Finberg, 2011). This result agrees with the findings reported by Marieb and Hoehn, (2013) and Galti *et al.* (2012) in Ethiopia. However, ferritin concentration increased among subjects within the age of 25 – 29 years and 35 – 39 years but decreased among subjects within the age of 40 – 44 years and 45 – 49 years. An increase in ferritin concentration among the subject could be as a result of high intake of food that contain iron while a decrease in ferritin concentration among the subject could also be as a result of inability of the subject to absorb iron (Sharp and Srai, 2007). This agrees with the report of Kozuki *et al.* (2012). The result of correlation analysis on the effect of age on haemoglobin concentration shows a negative correlation but positively correlated with ferritin concentration. This means that as the age of the subjects was increasing, their haemoglobin level was decreasing. This decrease was statistically significant ($p < 0.05$). However, ferritin concentration of the subject was increasing with an increase in age, although not significant ($p > 0.05$). this result is in accordance with the results of previous study done by Sanku *et al.* (2010).

Report from most developing countries shows that iron supplement intake during the time of pregnancy has limited effectiveness. This could be as a result of poor adherence, infection, inefficient health care systems and high rate of pre-existing anaemia (Alizadeh *et al.*, 2014).

Prevalence of anaemia among pregnant women in this study is high despite the fact that they received an average intake of routine drug. This finding is similar to that of Alizadeh *et al.* (2014) and Hemmininki and Rimpela (1991). The result of haemoglobin concentration of the subject who had the routine drug in this study shows a slight difference on intake of routine drug among pregnant women when compared to those that did not. Although there was a decrease in ferritin concentration of those that took the routine drug while there was an increase in ferritin concentration of those that are not regularly taking the routine drug. This could be that the subjects are already iron deficient before taking the routine drug. This result is in accordance with results as reported by Mcmahon, (2013) which said that the level of serum ferritin falls early in the development of iron deficiency and it is not affected by recent iron ingestion while an increase in the ferritin level of those that are not taking the routine drug could be as a result of the subjects taking other supplement apart from the one issued to them, although this result varies with the previous report of Arise, (2017). Intake of routine drug when correlate with haemoglobin concentration shows a negative correlation at ($p > 0.05$) but when correlate with ferritin concentration shows a positive correlation at ($p > 0.05$). This implies that increase in intake of routine drug seems to reduce the haemoglobin level but increase ferritin level among the subjects.

4.2 CONCLUSION

The study reveals that anaemia during pregnancy is highly prevalence. More than halve of the subjects was anaemic in both first, second and third trimester. This implies that children born by anaemic mothers stand the risk of being anaemic especially during exclusive breast feeding. Report obtained from previous studies have shown that iron reserve received by children as foetus during pregnancy runs out within 4 to 6 months after child birth thereby exposing infants to the risk of anaemia. Hence, public awareness campaign is recommended to create awareness on the need for early antenatal booking to provide opportunity for early detection and treatment of anaemia. There is a need to educate and implement strategies that will improve iron status of pregnant women. This will lead to sufficient placenta iron transfer to the unborn child.

4.3 Suggestions for Further Studies

- Iron status of pregnant women in urban area (Enugu Metropolis) should be carried out, and compared with the value settings across Enugu.
- Iron status of pregnant women and their newborn should be carried out, and compare the relationship between them.

- the feeding pattern of the pregnant women should be assessed alongside with their iron status, and check for any correlation.

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APPENDICES

CONSENT TO PARTICIPATE IN A RESEARCH

This consent form is intended for the study participant who is eligible to participate in this study. It may contain words that you do not understand and please ask the researcher to clearly explain any information that is not well understood. Please note, the term 'you' used in this form refers to the subject.

TOPIC: IRON STATUS OF PREGNANT WOMEN AT DIFFERENT TRIMESTER IN NSUKKA L.G.A, ENUGU STATE, NIGERIA.

You are asked to participate in this research study titled above, as part of my degree research project. Participating in this study is entirely voluntary and will be of help to this research. In order to decide whether you wish to participate in this research or not, you should understand enough about its risk and benefit to be able to make informed decision .

DESCRIPTION AND PURPOSE OF THE STUDY

You are invited to be part of this research study named iron status of pregnant women and other biochemical parameters. Pregnant women are particularly vulnerable to iron deficiency which is the most prevalent global nutrition deficiency and the most common cause of anemia worldwide. We believe that by determining the iron status of these pregnant women, it will help the scientific community on the correct approach in the treatment, prevention and management during pregnancy. The researcher expects to enroll pregnant women at different trimester, aged 20-40yrs as the research study participant.

STUDY PROCEDURE

If you volunteer yourself to be involved in this study:

1. You will give your basic information such as age, which trimester, Educational background etc.
2. You will be asked to provide a sample of blood (5ml) to determine your iron status (using serum ferritin), other haematological parameters (red blood count, haemoglobin level) and malaria parasite infection. Any leftover samples after the end of the analysis will be discarded at the end of the study.

POTENTIAL BENEFIT

The study will be of great benefit to you in the following ways:

1. Your involvement will help us in providing your iron status during the course of your pregnancy.

2. The result of the analysis will help in a better assessment tools and/or treatment and prevention for the study participant with iron deficiency during the course of their pregnancy

POTENTIAL RISK, DISCOMFORT AND INCONVIENCES

This project is not intended to provoke any emotional or physical discomfort. You may share confidential and sensitive information during interview. Hence, there may be discomfort in blood draw but we assured you of using experts to ensure little discomfort. There is no cost attached for participating in the study. The information collected/gotten may not be of benefit to you but should provide more general benefit to the society.

CONFIDENTIALITY AND PRIVACY

The study personnel are committed to respect your privacy. No other persons will have access to your personal health information or identifying information without your consent, unless required by law. Any medical record, documentation, laboratory samples or information related to you will be coded by study number to ensure that persons outside the study will not be able to identify you. The analysis conducted with your blood samples is being for research purposes ONLY and can only tell you your result based on request but not to any other person.

PARTICIPATION AND WITHDRAWAL

Your participation in this study is entirely voluntary. You can choose to or not to be a participant in this study. If you choose to participate in this study you can withdraw from the study at any time without any affect or loss of benefit to which you are entitled. your question will be satisfactorily and clearly answered. You will be adequately being informed of any information which might affect your condition or your willingness to continue participation in this study.

ALTERNATIVE TO PARTICIPATE

The alternative to participation is not to participate

IDENTIFICATION OF INVESTIGATOR, QUESTIONS AND COMPLAINTS:

If you have any question and complaints in regard to this research study, its procedure, risks and benefit or other issues patterning to it, please contact the principal investigators:

Uzoigwe kelechukwu chinaza, department of biochemistry; 08136500914 or through email; kelechukwuuzoigwe@mail.com

CONSENT

I acknowledge that the research study described above has been explained to me. It has been read to me or I have read the statements in this letter of information and procedures have been

explained to me during the consent discussion. I have the opportunity to ask questions about the study and any question I have asked have been answered to my satisfactory.

I authorized the use of my health information to the parties listed for the purposes described above. I voluntarily agree to participate in this study.

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Signature of subject date

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Signature of person conducting date

Informed consent discussion