ASSOCIATION BETWEEN DIETARY PATTERN, LIPID PROFILE AND ANAEMIA AMONG PREGNANT WOMEN ATTENDING ANTENATAL CARE UNIT, AMINU KANO TEACHING HOSPITAL, KANO, NIGERIA

BY

MUHAMMAD AUWAL SALIU

DEPARTMENT OF BIOCHEMISTRY,
FACULTY OF LIFE SCIENCES,
AHMADU BELLO UNIVERSITY,
ZARIA, NIGERIA

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BY

MUHAMMAD AUWAL SALIU,

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MAY, 2021

DECLARATION

I declare that the work in this dissertation entitled **"Association between Dietary Pattern, Lipid Profile and Anaemia among Pregnant Women Attending Antenatal Care Unit, Aminu Kano Teaching Hospital, Kano, Nigeria"**has been carried out by me in the Department of Biochemistry under the supervision of Dr. A. Salihu and Dr. S. B. Mada. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other institution.

Muhammad Auwal Saliu	_____	_____
Name of Student	Signature	Date

CERTIFICATION

This dissertation entitled "ASSOCIATION BETWEEN DIETARY PATTERN, LIPID PROFILE AND ANAEMIA AMONG PREGNANT WOMEN ATTENDING ANTENATAL CARE UNIT, AMINU KANO TEACHING HOSPITAL, KANO, NIGERIA" by MUHAMMAD AUWAL SALIU meets the regulations governing the award of the degree of Master of Science in Nutrition of the Ahmadu Bello University and is approved for its contribution to knowledge and literary presentation.

Dr. A. Salihu	_____	_____
Chairman, Supervisory Committee	Signature	Date
Dr. S. B. Mada	_____	_____
Member, Supervisory Committee	Signature	Date
Prof. A. B. Sallau	_____	_____
Head of Department	Signature	Date
Prof. S. A. Abdullahi_____	_____	
Dean, School of Postgraduate Studies	Signature	Date

DEDICATION

This work is dedicated to Almighty Allah for His endless favour, guidance and protection; May the peace and blessings of Allah be with Prophet Muhammad (SAW); to my beloved parents and wife for their inestimable support.

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My greatest gratitude goes to Allah who in His infinite compassion made this research work possible. May His peace and blessings be upon Prophet Muhammad, his families and companions. Iam expressing profound gratitude to my supervisors Dr. A. Salihu and Dr. S. B. Mada for their untiring effort, guidance, supports and encouragements towards the success of this research work. May Almighty reward you abundantly.

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ABSTRACT

Association between dietary pattern, lipid profile and anaemia among 112 randomly selected pregnant women in their second and third trimesters attending AKTH were assessed using standard methods. This study showed that 58.0% of the pregnant women investigated have attained tertiary level of education, 90.2% were married and 50.0% were fulltime housewives. Among the pregnant women, 56.4% were iron/folate supplement compliant. Moreover, the prevalence of anaemia, iron deficiency and IDA among the pregnant women during second trimester were 37.5%, 8.0%and 14.0% respectively, which significantly ($p<0.05$) increased to 65.8%, 12.9% and 21.2% respectively in the third trimester. Serum lipid profile revealed that the prevalence of dyslipidaemia, hypercholesterolaemia, hypertriglyceridaemia, high LDL and low HDL among the pregnant women during the second trimester were 69.6%, 19.6%, 36.6%, 18.8% and 49.1% respectively, which significantly ($p<0.05$) increased to 91.8%, 54.1%, 75.3%, 40.0% and 62.4% respectively in the third trimester.The pregnant women showed high risk of cardiovascular diseases in both second (81.0%) and third (85.0%) trimesters respectively. Furthermore, therewas a significant ($p<0.05$) positive correlation between serum iron and cholesterol, as well as transferrin saturation with cholesterol and low density lipoprotein irrespectiveof trimesters. In conclusion, pregnancy progression from second to third trimester is associated with increased risk for IDA, maternal dyslipidemia and cardiovascular diseases among the pregnant women.

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ABBREVIATIONS

ABU – Ahmadu Bello University

AI – Atherogenic Index

AKTH – Aminu Kano Teaching Hospital

CBC – Complete Blood Count

CHOL – Cholesterol

CHr – Reticulocyte Haemoglobin Content

DC – Daily Consumption

EDTA – Ethylene Diamine Tetra-acetic Acid

Hb – Haemoglobin

HDL – High Density Lipoprotein

ID – Iron Deficiency

IDA – Iron Deficiency Anaemia

LDL – Low Density Lipoprotein

MC – Monthly Consumption

RBCs – Red Blood Cells

SD – Standard Deviation

SF – Serum Ferritin

SI – Serum Iron

sTFR – Soluble Transferrin Receptor

TG – Triacylglycerol

TIBC – Total Iron Binding Capacity

TMB – 3, 3', 5, 5' – Tetra Methyl Benzidine

TS – Transferrin Saturation

VLDL – Very Low Density Lipoprotein

WC – Weekly Consumption

WHO – World Health Organisation

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Iron deficiency anaemia(IDA) is a global health problem and the most common nutritional disorder which accounts for approximately one-half of all anaemia cases (Johnson-Wimbley and Graham, 2011). The disorderinvolves diminished red blood cell production due to low iron stores in the body caused by inadequate intake, decreased intestinal absorption, increased demand and chronic bloodloss (WHO, 2008).

Iron is a trace element necessary for most cellular activities especiallythe syntheses ofhaemoglobin,myoglobinand cytochrome which makes it a crucial requirement for respiration,mitochondrial functionand energy production (Camaschella, 2017). Camaschella (2017) stated that iron is a component of iron-sulfur clusters and therefore indispensable for the prosthetic group of enzymes involved in cell proliferation, DNA repair andmany other functions. The most common cause of IDA is bleeding in the stomach and intestineswhich may be caused bycolonor stomach cancers, *hiatal hernia*, stomach ulcer, indiscriminateuse of non-steroidal anti-inflammatory drugs (Goddard *et al.,* 2000), or any other conditions/actions that can cause chronic blood loss. Other causes of IDA include swelling of the large intestine (colitis) or oesophagus, piles, polyps in the colon or intestines, endometriosis, celiac disease and intestinal surgery (Stein *et al.,* 2016). In women of childbearing age, heavy menstrual periods and pregnancy are the most common causes of IDA as the body requires additional iron for the foetus during pregnancy.

Similarly, iron is extremely important during pregnancy owing to rapid cell and tissue development associated with foetal growth (Burke *et al.,* 2014). During pregnancy, a net iron requirement of about 1200 mg is required (Breymann, 2015;Gambling *et al.,* 2011) which is mainly due to increment in

maternal erythrocyte mass (450mg), foetal development (250–300 mg), placenta (90–100 mg), general losses (200–250 mg) and blood loss on delivery equivalent to 150mg iron (300–500 ml blood loss) (Breymann, 2015). Despite the fact that iron expenditure is compensated by lack of menstrual blood loss during pregnancy, the final requirement is still high enough that iron recommendations during pregnancy (27 mg per day) far exceed (18 mg per day) those for non-pregnant and non-lactating women (Picciano, 2003). Since foetal iron requirements take precedence over maternal needs and storage (Gambling *et al.*, 2011), adequate iron intake is important to both mother and foetus.

Inadequate amount of iron during pregnancy could lead to IDA and its consequences includenegative perinatal outcomes such as premature labour, intrauterine growth restriction, low birth weight, birth asphyxia, neonatal anaemia, postpartum depression and developmental delays in baby's growth (Shill *et al.*, 2014; Zimmerman, 2007). Consequently, the World Health Organization (WHO) recommends daily oral iron and folic acid supplementation as part of antenatal care to decrease the risk of iron deficiency (ID), maternal anaemia and low birth weight (WHO, 2011). However, oral iron supplementation is challenged with poor compliance due to gastrointestinal side effects (Camaschella, 2017) and possibly limited knowledge on its significance.Breymann (2015) stated that lackofcomplianceisacommonproblem associated with oral iron supplementation and in Nigeria, only 21%ofpregnant women completed the dose of iron tablets during their last pregnancy (NPC and ICF, 2014).

Dietary pattern is referredto as the quantity, variety, or combination of different foods and beverage in a diet and the frequency with which they are routinely consumed.During pregnancy, there are no specific requirements for lipid consumptionand the requirement is similar to that of general population which should not be more than 30% of total calorie intake, with mono- and poly-unsaturated fatty acidsbeing the major components of consumed fats (FAO, 2010). Similarly,

alterations occur in lipid metabolism during pregnancy (Brizzi *et al.,* 1999); Kaaja (1998) reported that lipogenesis and fat storage are encouraged during anabolic phase of early pregnancy in preparation for rapid foetal growth in late pregnancy. Determination of lipid profile is greatly recommended during pregnancy so as to introduce rapid management approaches to prevent damaging effect of hyperlipidaemia associated with pregnancy (Pusukuru *et al.,* 2016). Thus, changes in serum lipid levels increase the risk of atherosclerosis and coronary heart disease (Yang *et al.,* 2015). Maternal atherogenic lipid profile in pregnancy has been implicated in perinatal morbidity and mortality (Vrijkotte *et al.,* 2012).

Iron deficiency is associated with the development of an atherogenic lipid profile and increased oxidative stress (Toescu *et al.,* 2002). On the other hand, anaemia dueto iron deficiency has been linked with elevated lipid accumulation in liver causedby low uptake of lipid as a result of impaired peripheral lipolysis or low uptake of chylomicrons and very low density lipoprotein remnants, making the lipoproteins to amass in the circulation (Ahmed *et al.,* 2012). Genomic studies reveal that in iron deficiency, there is elevated lipogenesis in the liver due to simultaneous increase in gene expression related to lipogenesis and decrease in gene expression related to β-oxidation (Davis *et al.,* 2012). Ahmed *et al.* (2012) reported that some changes in lipid metabolism may be related to effects of iron deficiency which are linked to key enzymes that depend upon iron as a cofactorsuch as carnitine acyl transferase.

1.2 Statement of Research Problem

Iron deficiency anaemia adversely affects maternal and foetal well-being throughout pregnancy, and is significantly associated with increased morbidity and foetal death (Noran and Mohammed, 2015). During pregnancy, IDA caused about 115,000 maternal deaths and 0.4% of disability adjusted life years (DALYs) (Black *et al.,* 2008; Stoltzfus *et al.,* 2004). Iron deficiency anaemia affects about52%

of pregnant women in the developing countries (Sato *et al.,* 2010) and even in the developed countries about 30% to 40% of pregnant women suffer from iron depletion (De Benoist *et al.,* 2008). Thus, increased iron demand is associated with pregnancy which further increases the risk of ID and IDA (Noran and Mohammed, 2015).

In Africa, 53.4% of pregnant women wereanaemic (Tandu-umba and Mbangama, 2015).In a study conducted among pregnant women in Sokoto State, Nigeria, the prevalence of anaemia was 21.3% whereas that of IDA based on ferritin level of <12.0 ng/ml and transferrin saturation of <16% was 13.5% and 7.69% respectively (Erhabor *et al.,* 2013). Likewise, 24.5% of pregnant women were anaemic in a study conducted in Kano State, Nigeria (Gwarzo and Ugwa, 2013), with only 2.3% of them completingtheir dose of iron tablets during their last pregnancy (NPC and ICF, 2014) which also signifies an increased risk of IDA.

Furthermore, most women of child bearing age do enter pregnancy with inadequate iron stores especially in developing countries which subsequently results in lowered iron stores in their foetuses and hence lead to an increased risk of subsequent IDA (Noran and Mohammed, 2015).In addition, gestational hyperlipidaemia contributes to an increased morbidity of preeclampsia and gestational diabetes (Jin *et al.,* 2016). It has also been reported from different studies that dyslipidaemia during pregnancy is associated with complications and adverse outcomes including gestational diabetes mellitus,gestationalhypertension, preeclampsia, intrahepatic cholestasis of pregnancy,induced preterm birth, babies large for their gestational age and macrosomia (Jin *et al.,* 2016; Pusukuru *et al.,* 2016; Vrijkotte *et al.,* 2012;Wiznitzer*et al.,* 2009).

1.3 Justification

Few studies with several inconsistencies have been reported on the relationship between IDA and serum lipid profilein women (Tiwari *et al.,* 2016). There is paucity of data on dietary pattern,

IDAand its relationship with lipid profile among pregnant women in Kano, Nigeria.Hence, this study seeks to investigate dietary pattern, IDA and their relationship with lipid profile among pregnant women in Kano, with the hope of generating evidenced-based data that can improve the quality of antenatal services offered to pregnant women in the study area.

Studying IDA among pregnant women and its relationship with lipid profile is crucial and will help strengthen the nutrition information system in the state and the nation at large. Thereisneed to monitor the progress made in meeting one of the Global Nutrition Targets (Reduce anaemia in women of reproductive age by 50%) by 2025 and Sustainable Development Goal 2 (Target 2.2) and 3 (Target 3.1). All these would help in making policies and programme-based decisions by government towards preventing upcoming consequences of IDA and dyslipidaemia for both mother and developing foetus.

In spite of the high prevalence of both IDA and dyslipidemia during pregnancy in Nigeria, the inter-relationship between the two diseases as well as the adverse consequences of their co-existence is not fully elucidated. Understanding the relationship would lead to a possibly paradigm shift in the current strategies for pregnancy management, especially in Africa.

1.4 Aim of the Study

The study aimed at investigating association between dietary pattern, lipid profile and anaemia among pregnant women in their second and third trimesters attending antenatal care unit, Aminu Kano Teaching Hospital (AKTH), Kano, Nigeria.

1.5 Specific Objectives

The present study seeks to:

i. assess the socio-demographic characteristics, medical/obstetrics history and consumption of iron- and lipid-rich foods among pregnant women at AKTH, Kano.

ii. evaluatethe prevalence of anaemia and IDA among pregnant women attending AKTH antenatal care unit.

iii. evaluate the prevalence of dyslipidaemia, changes in lipid profileand atherogenic index among pregnant women at AKTH, Kano.

iv. determine the association between dietary pattern, lipid profile and IDA among pregnant women at AKTH, Kano.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Anaemia in Pregnancy

Anaemia in pregnancy is defined as haemoglobin concentration of less than 11 g/dl in blood or haematocrit less than 33% at any point during pregnancy (WHO, 2001). Anaemia is of public health concern especially in developing countries which is associated with adverse outcomes in pregnancy (Black *et al.,* 2013). It is a frequently encountered medical disorder during pregnancy and causes serious concerns including adverse effects on the mother and foetus as well as contributing significantly to high maternal mortality in low-income countries (Sharma and Shankar, 2010). According to United Nation declaration 1997, anaemia is a major public health problem that needs total elimination,due to lack of functioning red blood cells (RBCs) produced in bone marrow which leads to the shortage of oxygen-carrying ability, initiating rare complications during lifespan (Sabina *et al.,* 2015). Pregnant women with haemoglobin concentrationof <11 g/dl at earlystage of gestation are termed anaemic; as pregnancy progresseshaemodilution occurs physiologically which may further increase the severity of anaemia (Chowdhury *et al.,* 2014).

World Health Organisation considered anaemia to be of public health importance based on several population studies thatshowed a prevalence of ≥ 5.0% while any prevalence that is up to 40.0% indicates severe public health problem (WHO, 2008).Anaemia is estimated to affect two billion people globally and approximately 56 million women worldwide with a global prevalence of 41.8% among pregnant women (Black *et al.,* 2013; Chathuranga *et al.,* 2014). Anaemia is more prevalent in low-income countries; however women from developed countries are also affected while pregnant mothers from both urban and rural areas tend to be vulnerable. The prevalence of anaemia varies from 53.8% to 90.2% among pregnant women in developing countries (De Benoist*et al.,* 2008). The prevalence was highest among pregnant women in South-east Asia (48.7%) followed by pregnant

women in Africa (46.3%) and lowest in Western Pacific with 24.3% (WHO, 2015). The Nigeria Demographic and Health Survey in 2018 reported a prevalence of anaemia among women aged 15-49 years and pregnant women to be 58.0% and 61.0% respectively (NPC and ICF, 2019). However, a lower prevalence of anaemia among pregnant womenwas reported to be 24.5% in Kano State, 21.3% in Sokoto State and 20.0% in Cross-river State (Erhabor *et al.,* 2013; Gwarzo and Ugwa, 2013; Okafor *et al.,* 2013).

Anaemia develops when there is lack of nutrients such as iron, vitamin B12 and folic acid needed for erythropoiesis or there is an increased loss of RBCs (Sabina *et al.,* 2015). In low and middle income countries, many factors causes anaemia during pregnancy such as deficiencies of micronutrient (iron, folate, and vitamins A and B12), chronic infections which include tuberculosis and human immunodeficiency virus as well as parasitic infections such as hookworm infestations and malaria (McClure *et al.,* 2014; Okube *et al.,* 2016; Ononge *et al.,* 2014). Other major causes of anaemia include; acute or chronic blood loss, chronic diseases (like renal disease and neoplasia), haemolytic anaemia (caused by drug or congenital) and haemoglobinopathies like sickle cell and thalassemia (WHO, 2001). During pregnancy, contributions of each factor causing anaemia depend on dietary pattern, geographical location and season (Stephen *et al.,* 2018). However, the leading cause of anaemia among pregnant women in West Africa is reported to be inadequate intake of diets rich in iron (McClure *et al.,* 2014; Ononge *et al.,* 2014) and consumption of non-haem iron sources which have low bioavailability as well as intakeof iron sources together with iron inhibitors.

Anaemia is an essential risk factor which is associated with an increased occurrence of maternal and foetal morbidity and mortality (Anlaakuu and Anto, 2017). During pregnancy,anaemia has been reported to influence negative maternal and child health effect as well as increasing the risk of perinatal mortality (Mbule *et al.,* 2013). It also contributes to an increase in low birth weight, still birth and foetal wastage (Anlaakuu and Anto, 2017). About 3.0% of maternal deaths in Africa are

directly attributable to anaemia (Khan *et al.*, 2006). Bondevik *et al.* (2000) reported that during pregnancy, anaemia causes reduction in tolerance to blood loss which subsequently leads to impaired function and cardiac failure. The adverse health effects of maternal anaemia include increased risk of cardiac diseases, fatigue, impaired immune function and poor work capacity (Mbule *et al.*, 2013; Stevens *et al.*, 2013). Kozuma (2009) reported that retrospective studies in developing countries have shown that the frequencies of low birth weight, premature delivery and foetal deaths are significantly greater in cases of maternal anaemia.

2.2 Iron Metabolism and Requirements during Pregnancy

2.2.1 Iron metabolism

Iron is one of the essential micronutrients for humans, owing to its vital role in key biological processes (Burke *et al.*, 2014). Tissue oxygenation is one of the crucial roles accomplished by RBCs which requires haemoglobin for its generation; and iron is a fundamental component of haemoglobin (Miller, 2013). Iron requirements are increased in conditions that need more surge in RBCs like the increased tissue mass of a growing foetus (Burke *et al.*, 2014). Iron absorption is principally controlled and regulated inside the intestine, there is no mechanism of excretion from the renal or hepatic tissues after iron is absorbed (Andrews, 2008) which makes iron homeostasis closely and tightly regulated. Iron is maintained in aqueous circulation in order to be transferred to other cells after absorption and this is possible for the body system by storing it in a non-reactive state inform of transferrin or ferritin in body cells (Andrews, 2008). Iron stored as ferritin within cells could be situated in the mitochondria, cytoplasm or nucleus (MacKenzie *et al.*, 2008). Ferroportin and divalent metal transporter 1 (DMT1) are responsible for exporting and importing iron across cell membranes respectively (Miller, 2013). Hepcidin, a protein secreted primarily by hepatocytes into

the circulation binds ferroportin which prevents its function thereby lowering the concentration of serum iron as illustrated in Figure 2.1.

Furthermore, as levels of hepcidin decreases, intestinal iron absorption increases coupled with transport of iron from the hepatic stores (Rehu *et al.,* 2010). Hepcidin levels in the foetus can change maternal responses and iron pathways which are interconnected through the placenta (Kling and Coe, 2016). Figure 2.2 illustrates that maternal hepatic hepcidin exerts negative feedback inhibition of iron absorption and lowers iron transport via blockage of macrophage iron release in addition to shunting to the liver and placental transfer to the foetus as described by Kling and Coe (2016). Likewise, in a state of foetal iron abundance or inflammation, the foetal liver hepcidin can down-regulate placental delivery (Gambling *et al.,* 2009). Maternal hepcidin concentration usually reduce during pregnancy in order to meet the six-fold greater needs for iron absorption (O'Brien *et al.,* 2003) which facilitate placental transfer through apical transferrin receptor importer and basolateral ferroportin exporter on placental syntrophoblasts (Rehu *et al.,* 2010). High levelsof foetal or maternal hepcidin can hinder placental iron transferand leads to lower foetal iron concentrations (Dao *et al.,* 2013; Dosch *et al.,* 2016). Severe placental malfunction that can cause intrauterine growth restriction may also limit placental iron transfer notwithstanding the low levels of iron in the foetus (Briana *et al.,* 2013).

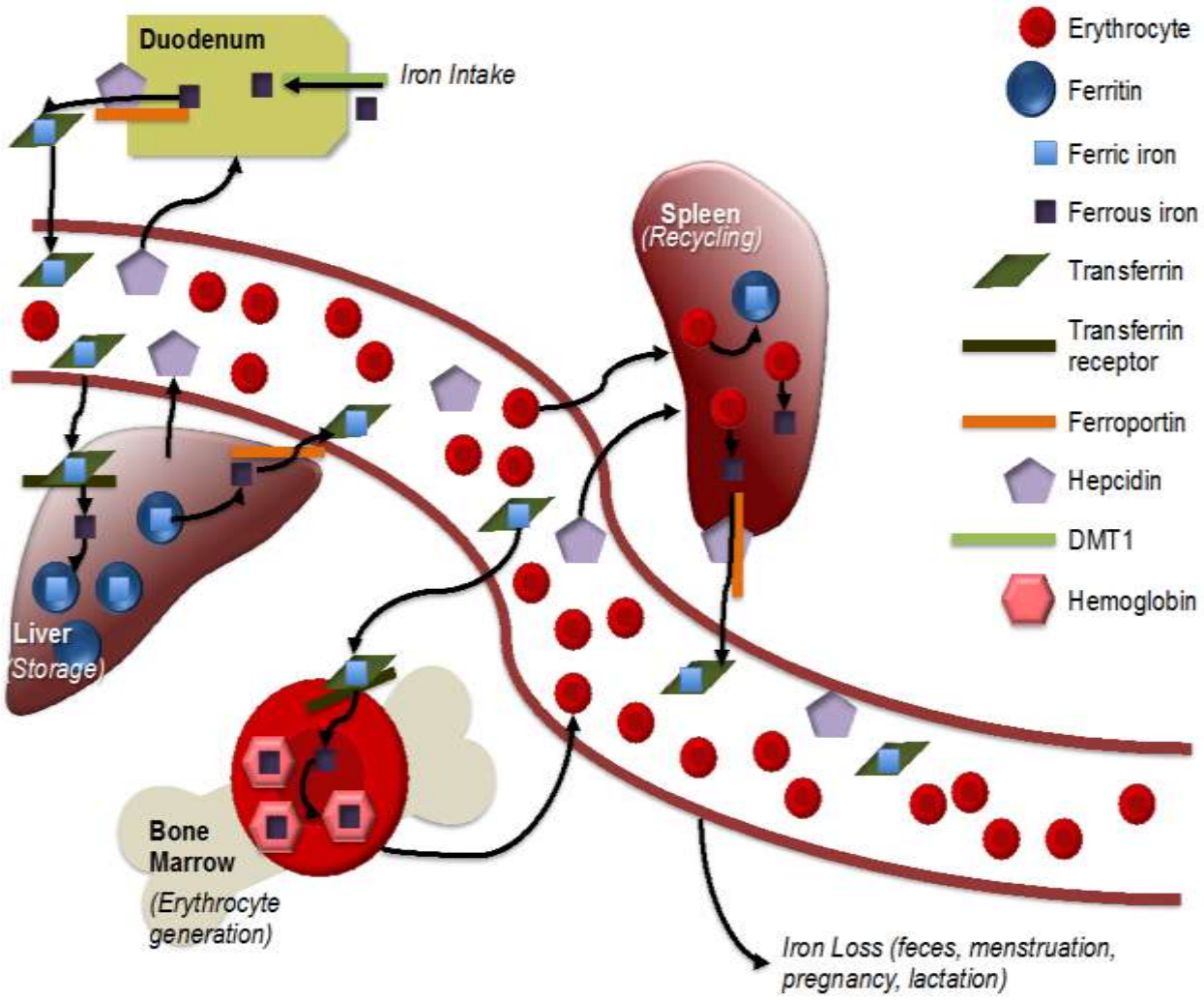


Figure 2.1: Basic illustration of iron metabolism(Adapted from Escobar-Morreale, 2012)

Iron is absorbed within intestine, with ferric iron being imported by DMT1. Ferrous iron is converted to ferric iron which is now exported by ferroportin. Iron bound to transferrin is imported into the liver, heart and other storage sites which are retained in ferritin, utilising the transferrin receptor. Hepcidin aids in controlling iron metabolism by binding to ferroportin, thereby inhibiting iron export. In the bone marrow, iron is integrated into haemoglobin for incorporation into RBCs, while in the spleen macrophages generally recycle iron from RBCs. There is no mechanism for iron excretion by the renal or hepatic tissues, however small amounts are lost via faeces. Menstruation, pregnancy and lactation lead to iron loss in women.

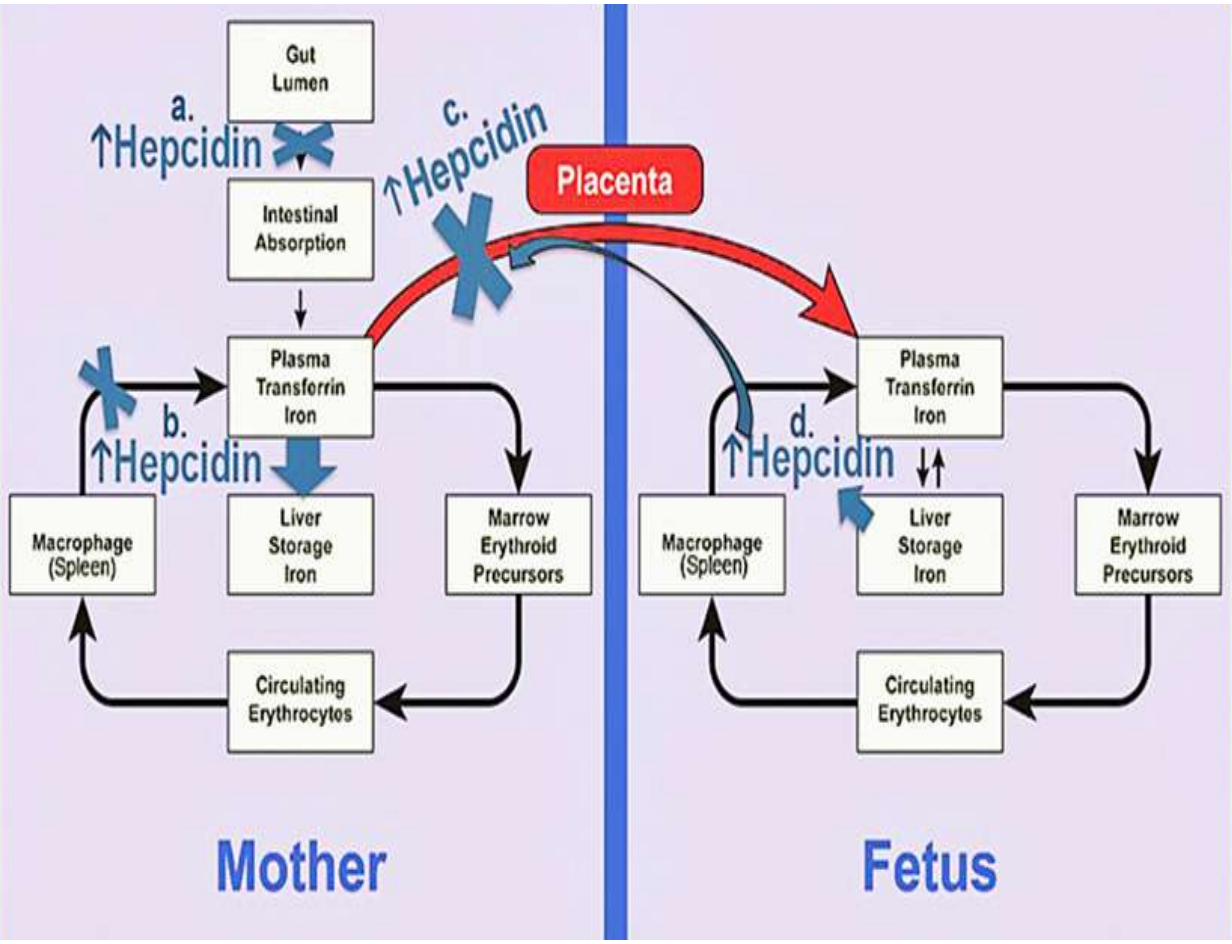


Figure 2.2: Maternal-foetal iron transport(Adapted from Kling and Coe, 2016)

Iron uptake by the foetus can be inhibited at several points. Increased hepcidin concentrations can block; (a.) the six-fold higher rates of absorption through maternal gut lumen, (b.) macrophage export and shunting to the liver, and (c.) placental transfer to the foetus. Similarly higher hepcidin levels can (d.) lower delivery in foetal iron abundance or inflammation (reverse arrow).

2.2.2 Iron requirements in pregnancy

Women of reproductive age should have around 2000 mg iron in the body system, with 60-70% found in RBCs; while the remaining is stored in the liver, spleen, and bone marrow (Kozuma, 2009), besides the demand for iron increases once a woman becomes pregnant. One of the most significant micronutrient particularly during pregnancy is iron owing to rapid cell and tissue development involved in foetal growth (Burke *et al.,* 2014).

Consequently, an additional 50% of the amount of iron present in non-pregnant state should be ingested during pregnancy which tallies to an extra intake requirement of 4 mg iron per day (Kozuma, 2009). Since the absorption of iron in food is about 10%, the additional oral iron intake necessary for a pregnant woman is calculated to be approximately 40mg/day. Thus, 600-800 mg of iron is required during pregnancy (Gambling *et al.,* 2011) where about 300 mg of iron is required by the foetus, 25 mg by the placenta and about 500 mg for the increased volume of RBCs (Bothwell, 2000). There is an equivalent saving of 150 mg of iron due to amenorrhea assuch approximately 850 mg of additional iron is required during pregnancy (Sharma and Shankar, 2010). These requirements of 800-850 mg of iron linked with pregnancy is beyond and exceed the 230 mg of iron that a woman would have required even though she is not pregnant, inclusive of the 150 mg that she may lose via blood loss during delivery (Beaton, 2000). Although this iron requirement is compensated by absence of menstrual blood loss during pregnancy, the net cost remains high enough that iron recommendations during pregnancy is far beyond that of non-pregnant and non-lactating woman (Picciano, 2003). Figure 2.3 indicates the requirements for iron during pregnancy.

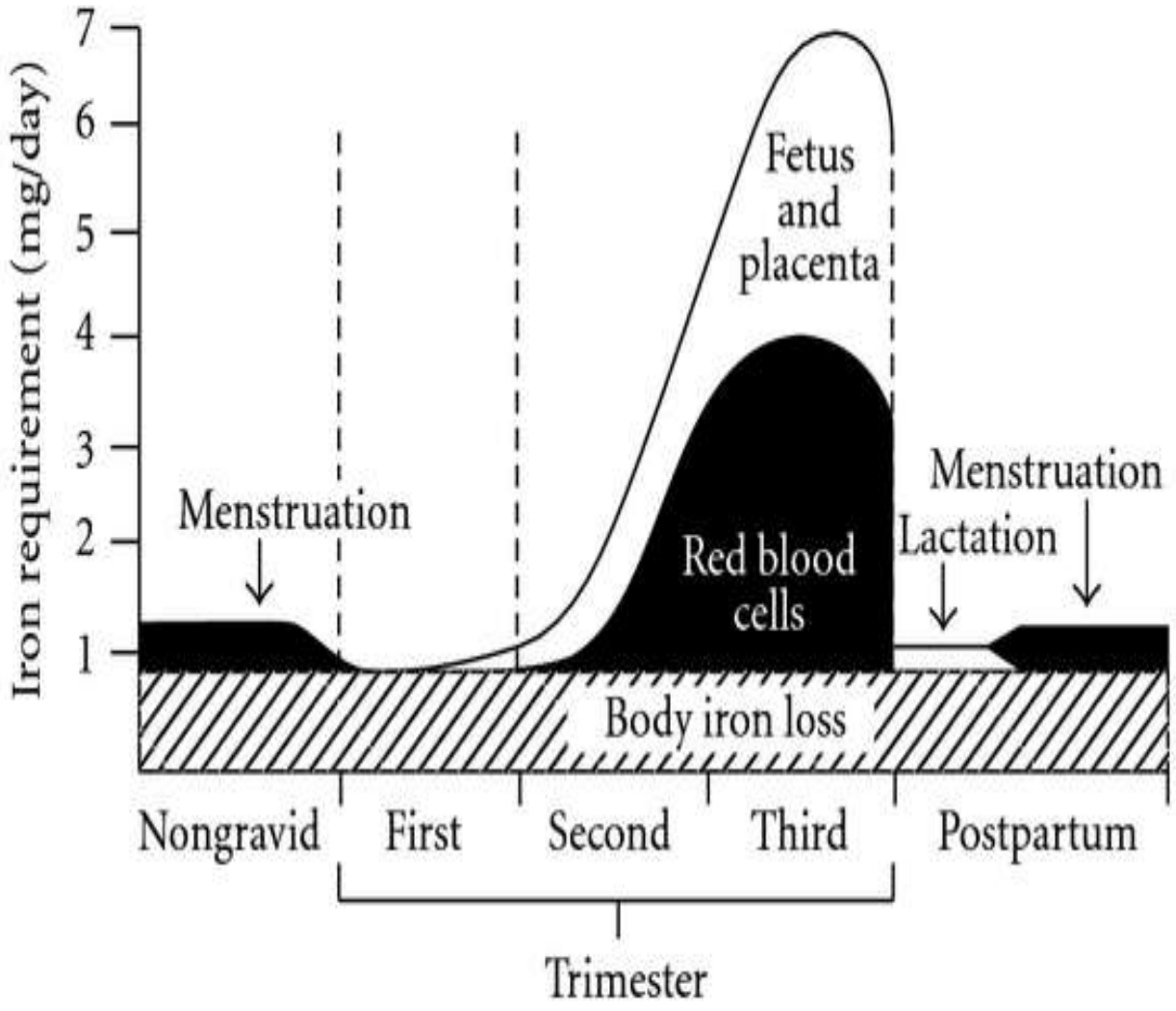


Figure 2.3: Requirements for absorbed iron in pregnant and lactating women(Adapted from Bothwell, 2000)

In addition, foetal iron requirements take preference over mother's needs and storage (Gambling *et al.,* 2011) and therefore sufficient iron consumption is imperative to both mother and foetus. The additional iron cannot be met by diet alone and the stores which have about 500 mg of iron get depleted in pregnancy (Sharma and Shankar, 2010) as such iron supplementation is recommended.

Iron nutritional status relies on long-term iron balance which is favoured by consumption of sufficient amounts of iron in the diet or through iron supplementation. This iron balance is unfavourably affected by loss of iron through menstruation, intestinal mucosal turnover and excretion, skin desquamation and lactation (Bader *et al.,* 1996). Absorption of iron is normally 15-30% (haem iron) which increases to 50% in iron deficient state; moreover the absorption reduces to 5-8% with an excessive haem diet (Sharma and Shankar, 2010) because its absorption is typically not affected by inhibitors. However, absorption of non-haem iron found in other iron sources such as vegetables, cereals, seeds, milk and eggs can be improved by enhancers (haem, proteins, ascorbic acid and fermentation) and lessened by inhibitors like phytic acid, fibre, calcium, tannins, tea, coffee, chocolate and herbal infusion (Akkermans *et al.,* 2016; O'Brien*et al.,*2003).

2.3 Iron Deficiency Anaemia in Pregnancy

Iron deficiency anaemia refers to reduced red blood cell production owing to decrease iron stores in the body and it is the most common nutritional disorder globally which accounts for about 50% of anaemia cases (Johnson-Wimbley and Graham, 2011). During IDA, there is incomplete haemoglobin synthesis which leads to microcytic and hypochromic erythrocytes (AlDallal, 2016). This results in diminished ability of blood to deliver oxygen to other body cells and tissues due to insufficient haemoglobin (Akodu *et al.,* 2016; Cook *et al.,* 1994). Iron deficiency still remains the most common deficiency in women of reproductive age worldwide, which is more common in pregnancy due to higher iron demand (Akodu *et al.,* 2016).

Estimates from WHO, shows that prevalence of IDA in pregnancyranges from 14% in developed countries to 56% in low and middle income countries (WHO, 2015). Zamani *et al.* (2008) stated that in low income countries, more than 40 million pregnant women suffer from iron deficiency and its consequences. Iron deficiency anaemia is associated with 75-95% of anaemia cases during pregnancy (Halimi *et al.,* 2011) and the high occurrence of this condition in developing countries among pregnant women has considerable health consequences and economic cost implications (Osungbade and Oladunjoye, 2012).

2.3.1 Causes of iron deficiency anaemia in pregnancy

Risk factors associated with IDA in pregnancy include; iron deficiency prior to pregnancy, multiple pregnancies, short recovery between pregnancies, poor nutritional status, multiparityand poor socio-economic status (Breymann, 2013). Other major risk factors for decrease iron levels include low consumption of bioavailable iron, infections (such as malaria, helminthic infections), teenage pregnancy and ethnic or cultural practicesthat interfere with iron intake and/or absorption (Cao and O'Brien, 2013; Milman, 2011). Two major factors that contribute to development of IDA during pregnancy include; the mother's iron stores at the time of conception (usually low store) and the quantity of iron absorbed during gestation (Osungbade and Oladunjoye, 2012). Pregnant women are principally susceptible to iron deficiency, not merely due to the large amount of iron needed for foetal and placental development (Beaton, 2000; Bothwell, 2000), but owing to the fact that several pregnant women enter pregnancy with inadequate iron stores especially in low and middle income countries (Milman, 2011). McMahon, (2010) also reported that regular occurrence of anaemia during pregnancy in developing countries is an indication that iron stores are often insufficient and physiological adaptations to pregnancy are inadequate to meet the higher demands. In developing countries, other factors linked with IDA are poverty, malnutrition, and famine (Camaschella, 2015).

In various countries such as India, vegetarianism may perhaps be common because of religious beliefs which render pregnant women to be more vulnerable to the development of iron deficiency due to intake of non-haem iron from plants; while haem iron is more absorbed efficiently (Burke *et al.,* 2014). Iron levels can also be affected by the consumption of other nutrients that may enhance or inhibit iron absorption (Collings *et al.,* 2013). Psychological and social factors could also increase the risk of IDA during pregnancy through reduced adherence and compliance to iron supplementation (Tran *et al.,* 2013). Access to healthcare and food security play vital roles in development of IDA during pregnancy,and other factors to be considered include the existence of anaemia control programs (like universal supplementation), fortification policies, economic situation and agricultural productivity of localities (Pasricha *et al.,* 2013).

2.3.2 Consequences of iron deficiency anaemia in pregnancy

The consequences of iron deficiency in pregnant mothers are multifaceted and have adverse effects for both mother and the foetus. Low iron intake and low stores during pregnancy lead to anaemia, it is also associated with tiredness, weakness, reduced immune response and decreased cognitive performance (Milman, 2011). Iron deficiency can as well increase the risk of delivery complications and perinatal maternal deaths (Milman, 2011). It has been significantly associated with increased perinatal mortality.

Foetal brain development might be at high risk when iron requirements are inadequate (Lozoff and Georgieff, 2006) and studies have shown the reverse effect of iron deficiency in pregnancy on motor and intellectual development of children (Chang *et al.,* 2013; Tran *et al.,* 2013). Maternal iron deficiency has been linked with cognitive and behavioral deficits owing to the importance of iron to foetal brain development (Chang *et al.,* 2013; Milman, 2011), which is likely facilitated via decreased iron stores during birth and can subsequently lead to iron deficiency in the infant (Cao and

O'Brien, 2013). It has been reported that prenatal iron deficiency anaemia in pregnancy has negative adverse effects on child cognitive development and is significantly associated with the child's mental development (Tran *et al.,* 2013). Iron deficiency in pregnancy may increase the risk of iron deficiency anaemia during lactation (Picciano, 2003).

Additionally, Zeng *et al.* (2008) reported a reduction in early neonatal mortality upon prenatal supplementation with iron- folic acid in a study conducted in China. Milman (2011) reported that maternal iron deficiency has been identified as a risk factor for small-for-gestational-age, neonatal deaths and preterm delivery. Several studies have demonstrated that an association exists between iron deficiency anaemia, pre- term birth and low birth weight (Abu-Saad and Fraser, 2010; Haider *et al.,* 2013; Levy *et al.,* 2005). It was revealed that maternal anaemia among pregnant women is a risk factor for pre- term delivery and low birth weight with odds ratio of 1.2 and 1.1 respectively evidenced from a retrospective population- based study (Levy *et al.*,2005). Similarly, a meta- analysis reported that cohort studies showed a greater risk of pre- term birth and low birth weight with anaemia in the first or second trimester of pregnancy (Haider *et al.,* 2013).

2.3.3 Diagnosis of iron deficiency anaemia

Diagnosis of iron deficiency anaemia requires laboratory investigation and must be ascertained via laboratory testing including evidence of decreased iron stores (US PSTF, 2006). Iron deficiency needs to be differentiated from other causes of anaemia due to its relation with underlying diseases that require certain investigation (Pasricha *et al.,* 2010). Iron deficiency diagnosis presents with a lot of difficulty and therefore several combinations of indicators are required to accurately assess the deficiency (Lopez *et al.,* 2016).

The initial analysis of anaemia involves the use of simple methods (Dignass *et al.,* 2015) which include a complete blood count (CBC), peripheral blood smear, reticulocyte count, and serum iron

indices (AlDallal, 2016). The full blood count aids in estimating the mean corpuscular volume, which determines the average size of erythrocytes and mean corpuscular haemoglobin concentration that determines the concentration of haemoglobin in a certain amount of packed erythrocytes (AlDallal, 2016). Red cell indices on CBC show decreased mean cell volume that tallies with microcytosis and decreased mean cell haemoglobin conforming to hypochromia during iron deficiency (Zeng *et al.*, 2018). Mean cell haemoglobin and volume are easily accessible, sensitive and inexpensive indicators, as they turn out to be abnormal only when iron deficiency is long-lasting (Zeng *et al.,* 2018). The classical features of iron deficiency anaemia include microcytic and hypochromic RBCs as well as low iron stores. While microcytic anaemia is described by small erythrocytes and iron deficiency, about 40% of patients with iron deficiency anaemia may have normocytic erythrocytes (Johnson-Wimbley and Graham, 2011; Shander *et al.*, 2011).

Furthermore, red cell distribution width is an alternative marker used in combination with mean corpuscular volume to distinguish between mixed causes of anaemia and that of a single cause (AlDallal, 2016). High value of red cell distribution widthindicatesearly stage of folate and iron deficiency anaemia as well as change in the size of erythrocytes which leads to macrocytic anaemia (Johnson-Wimbley and Graham, 2011; Northrop-Clewes and Thurnham, 2013).

Serum ferritin measurement is widely used for determining the iron status and it is the most sensitive and specific test for identifying iron deficiency (Lopez *et al.,* 2016); however it is challenged, as its levels increase in inflammatory conditions, liver disease or malignancy. It is the most universal and suitable index of iron deficiency (Pasricha *et al.*, 2010). Serum ferritin level is used as the surrogate marker for iron deficiency though serum ferritin is an acute phase reactant and could be elevated in cases of inflammation or infection, therefore a simultaneous analysis of inflammatory markers such as C-reactive protein is worthwhile in condition of anaemia with increased ferritin to eliminate reactive causes (Hansen and Hansen, 1986; Khalafallah and Dennis, 2012). Iron deficiency is

probably not present when serum ferritin level is greater than 100μg/L (Goddard *et al.,* 2000). Previous study showed that when the level of ferritin increases to 30μg/L, the diagnostic precision and accuracy would be improved and the sensitivity increases from 25 to 92% accordingly (Mast *et al.,* 1998). Ferritin levels equal or greater than 50μg/L can still be classified as iron deficient for patients with acute and chronic inflammatory disorders, malignant and liver diseases (Guyatt *et al.,* 1992; Kassebaum *et al.,* 2014).Patients with chronic renal disease and in situation of haemodialysis are classified as iron deficient if their ferritinlevels are100μg/L and 200μg/L respectively (Locatelli *et al.,* 2004; Thomas *et al.,* 2013).

Serum soluble transferrin receptor (sTfR) is an alternative and suitable biomarker in the diagnosis of iron deficiency which is not affected by inflammation (Zeng *et al.,* 2018). It is an additional test for diagnosing IDA and indirect method of measuring erythropoiesis which is elevated in patients with iron deficiency (Koulaouzidis *et al.,* 2009). Synthesis of transferrin receptors increases in patients with iron deficiency which subsequently leads to elevation of sTfR (Mast *et al.,* 1998). In patients with diseases affecting erythropoiesis (such as chronic lymphocytic leukaemia and haemolytic anaemia), their levels of sTfR are elevated which stands as a limitation (Thomas *et al.,* 2013). It has the advantage of not being affected by inflammatory conditions likewise identifying IDA in patients with anaemia of chronic disease (WHO, 2004). Another indicator is the transferrin saturation in which low level (< 16%) shows iron deficiency although an increase to 20% in patients with inflammation still signifies iron deficiency (Lopez *et al.,* 2016).

A recent biomarker for diagnosing iron deficiency is bone marrow aspiration which is highly specific and not affected by inflammation, although not used often except other tests are false or conflicting (Zeng *et al.,* 2018). Bone marrow aspiration is also not convenient because of relative high cost and it is affected by recombinant human erythropoietin (Lopez *et al.,* 2016). Despite the fact that analysis of bone marrow iron stores is commonly considered as the ultimate marker of iron deficiency, it is

challenged with impracticability and the invasive nature (Khalafallah and Dennis, 2012). Absence of stainable iron in bone marrow biopsy (which is considered a standard diagnostic measure) signifies iron deficiency (Karagulle *et al.,* 2013). Furthermore, studies have shown that estimation of reticulocyte haemoglobin content (CHr) is recognized as an indirect measure of the functional iron attainable for new erythrocyte synthesis (Mast *et al.,* 1998). Mast *et al.* (1998) reported that CHr of <28 pg/L had an optimal sensitivity of 74% and specificity of 73% for diagnosing IDA. Thomas *et al.* (1996) also reported that functional iron deficiency was defined as CHr of <28 pg/L and this makes CHr in combination with other parameters a highly useful and reliable safe method for diagnosing iron deficiency anaemia (Bermejo and Garcia-Lopez, 2009).

Hepcidin plays a vital role in iron metabolism and is mainly synthesized in the liver which is later secreted into the blood circulation. Hepcidin is excreted via kidney hence, it can be detected and measured in urine (Nemeth and Ganz, 2009; Weinstein *et al.,* 2002). Hepcidin circulates within ferroportin in the plasma and responds to several stimuli that controls serum iron and iron stores (Nemeth and Ganz, 2009). Previous studies established the fact that hepcidin levels are decreased in iron deficiency (Nemeth and Ganz, 2009; Weinstein *et al.,* 2002). The estimation of blood and urine hepcidin levels can be attained via mass spectrometry and immunoassays (Kemna *et al.,* 2007). However, the utilization of serum hepcidin for diagnosis in iron deficiency has not been well-defined in clinical application (Pasricha *et al.,* 2011). Thus, measurement of hepcidin stands to be a prospective precise and accurate test that would reveal the actual iron status with fewer limitations (Khalafallah and Dennis, 2012).

Moreover, a more accurate diagnosis of IDA is the measurement of soluble transferrin receptor and serum ferritin (Guyatt *et al.,* 1992; Hansen and Hansen, 1986; Punnonen *et al.,* 1997) however, transferrin receptor is not a well-standardized test which could be reliably replicated with high accuracy and precision in most laboratories globally (Punnonen *et al.,* 1997). The generally

obtainable and accessible laboratory analyses that estimate iron status include serum iron, transferrin, total iron-binding capacity, transferrin saturation and serum ferritin which are extensively and universally utilised in clinical practice (Guyatt *et al.,* 1992; Hansen and Hansen, 1986). Patients presumed to be having iron deficiency anaemia are to undertake iron analysis and the outcomes are correlated with that of red cell indices. Iron investigations indicating iron deficiency anaemia comprise of low haemoglobin (<13 g/dl, 12 g/dl in women and 11g/dl in pregnant women), low serum ferritin (<30 μg/l), high total iron-binding capacity (>13.1 μmol/l) and low transferrin saturation (<15%) (Bermejo and Garcia-Lopez, 2009; Clark, 2009). Serum iron concentration has substantial diurnal variation and tends to be low in inflammation and iron deficiency anaemia, therefore must not be used alone as a basis for diagnosis of iron deficiency (Pasricha *et al.,* 2011).

In diagnosing iron deficiency anaemia, it is essential to consider the entire picture rather than depending on a single test result. Diagnosing iron deficiency for patients with inflammation is challenging and also cannot be determined on the basis of a single test (Zeng *et al.,* 2018). Recommendation of further assessment of likely underlying causes of blood loss should also be considered for the patients with IDA (AlDallal, 2016). Overall, recent technologies such as hypochromic reticulocytes and reticulocyte haemoglobin testing, sTfR and hepcidin are reported to be developed with improved specificity, sensitivity, reproducibility, and cost effectiveness (Pasricha *et al.,* 2011; Zini *et al.,* 2011).

2.3.4 Prevention of iron deficiency anaemia during pregnancy

2.3.4.1 *Ironsupplementation*

World Health Organisation recommends daily oral iron and folic acid supplementation as part of the antenatal care to reduce the risk of maternal anaemia, iron deficiency and low birth weight (WHO, 2012). Alternating iron supplementation (1-3 times weekly) seems to be as effective as daily

supplementation for preventing anaemia during pregnancy (Breymann, 2013). In the course of second and third trimesters of pregnancy, a daily dose of 4-6 mg of iron is needed (Bothwell, 2000) which is challenging to meet through usual diet, particularly in low income countries where most foods have inadequate bioavailable iron, therefore iron supplementation during pregnancy has been reported to be of immense benefit (Bodnar *et al.,* 2002; Milman *et al.,* 2005).

Different forms of preventive treatment of iron deficiency anaemia in pregnancy exist and involve iron supplements given either orally or parenterally, it can as well be administered in form of blood transfusion and recombinant erythropoietin with iron (Osungbade and Oladunjoye, 2012). Oral iron is the first choice in the preventive treatment of iron deficiency anaemia for pregnant women because of its low cost, safety, and effectiveness (Bencaiova *et al.,* 2009). Iron fumarate or sulphate, is one of the most common prophylaxis used for prevention of iron deficiency and iron deficiency anaemia in pregnancy (Osungbade and Oladunjoye, 2012). Iron supplementation has been widely used in low and middle income countries as an intervention for prevention of iron deficiency anaemia in pregnancy (Peña- Rosas and Viteri, 2006). The International Nutritional Anaemia Consultative Group recommends daily intake of iron (60 mg) for non-anaemic pregnant women when intake for above six months is possible before childbirth (Stoltzfus and Dreyfuss, 1998). Stoltzfus and Dreyfuss (1998) proposed an additional daily intake of 120 mg of iron among non-anaemic pregnant women when the length of intake is shorter or in regions where pregnant women are usually anaemic, likewise when the prevalence of iron deficiency among women is high.

2.3.4.2 *Ironfortification*

Iron fortification of foods is another approach for prevention of iron deficiency anaemia during pregnancy in low income countries. Food fortification with iron could be useful and cost effective in areas where access to oral iron supplementation among pregnant women is highly challenging (Osungbade and Oladunjoye, 2012). Furthermore, iron fortification would be essential in low income

countries where the rate of compliance with iron supplementation is low (McMahon, 2010). There has been iron fortification of various foods such as salt, maize and wheat flour, sugar, beverage, noodles, milk and rice that has been successfully utilized to boost iron stores, and therefore increase haemoglobin concentration in the population (WHO, 1998). Multiple micronutrient powders comprising of iron could as well serve as a preventive measure for iron deficiency during pregnancy (Burke *et al.*, 2014).

2.3.4.3 *Dietary diversification*

Dietary diversification interventions are interventions that change food consumption at the household level, such as increasing the consumption of animal-source foods (Gibson and Anderson, 2009). In most resource-poor settings, starch-based diets with limited access to meats, dairy, fruits, or vegetables, are the dominant diets which are good sources of iron. The aim of changing household diet is to increase the variety and quantity of iron/micronutrient-rich foods including animal-source foods so as to decrease iron and micronutrient deficiencies(Nair*et al.*,2016). Dietary diversification among pregnant womenand other vulnerable population is generally achieved through social and behaviour change activities as well as nutrition education, but can also include increased production of nutrient-rich foods and improved availability and access to diverse foods.

Food-based strategies to improve iron status involve increased dietary diversity and traditional food-processing techniques aiming at increasing the total iron content and improving the dietary absorption of non-haem iron in the diet of pregnant women and individuals that are deficient. Food-based approaches are sustainable ways to improve iron status and should always be the first priority (Andersson and Hurrell, 2010). Such strategies can be implemented at the household level, are safe and do not require universal screening for iron deficiency.

2.3.5 Treatment of iron deficiency anaemia in pregnancy

When anaemia has been diagnosed during pregnancy, it might be difficult to predict the progression of the condition and whether it will worsen; besides maternal and foetal risks increase as anaemia becomes more severe (Breymann, 2013). Certain factors that should be considered when deciding on the treatment approach to use for iron deficiency anaemia include; the severity of anaemia, time remaining before delivery, maternal comorbidity, additional risks such as premature labour and the patient's willingness to receive treatment (Breymann, 2013). The most common treatment options for iron deficiency anaemia are supplementation with oral or parenteral iron, stimulation of haemopoiesis with growth factors like recombinant human erythropoietin and the administration of heterologous blood (Bashiri *et al.*, 2003; Krafft *et al.*, 2009; Munoz *et al.*, 2008).

Supplementation with oral iron remains the gold standard for the treatment of mild to moderate iron deficiency anaemia. Studies are still ongoing to confirm whether intermittent or weekly intake of oral iron has advantage over daily oral iron intake or its equivalent (Khalafallah and Dennis, 2012). Dosage of 100-200 mg iron per day is agreeable between the ideal haemoglobin increase and the tolerability of the iron (Breymann, 2013). However, oral iron therapy is challenged with side effects such as gastrointestinal disturbances characterized by colicky pain, constipation, diarrhoea, heartburn, nausea and vomiting which occur approximately in 50% of patients taking the oral iron preparations (Cancelo-Hidalgo *et al.*, 2013; Galy *et al.*, 2010; Sharma, 2001). In such situations, dose is either reduced or a different product is tried (Khalafallah and Dennis, 2012; Mahomed, 2000). Another challenge is the lack of compliance with the daily intake of the oral iron as it was reported that merely 36% of pregnant women take oral iron regularly, despite their level of awareness on the complications of iron deficiency (Breymann, 2013).

Other potential factors that could challenge the effectiveness of oral iron therapy includes; presence of infection that suppresses erythropoiesis, complicating disorders such as kidney failure,

gastrointestinal diseases like Crohn's disease and ulcerative colitis, malabsorption of iron like celiac disease, drugs that inhibit erythropoiesis such as immunosuppressants and cytotoxic agents, hemorrhage and inappropriate diagnosis of iron deficiency (Breymann, 2013). Furthermore, the most common prescribed oral iron is primarily composed of ferrous salts (Kumar *et al.,* 2005) which have been characterized with low and variable absorption rates. Its absorption can further be restricted by mucosal luminal damage likewise ingestion of certain foods (Sharma, 2001). Therefore, ferric compounds were introduced to avoid such difficulties which also present with less solubility and poor bioavailability (Geisser, 1990). The most commonly used iron (II) compound is iron (II) sulfate; others include iron (II) complexed with succinate, gluconate and fumarate.

Parenteral iron serves as the main alternative to oral iron therapy. The conditions which necessitates parenteral iron administration includes; inadequate or no response to oral iron, inadequate absorption of oral iron due to intestinal disease, severe anaemia, requirement for rapid efficacy, poor compliance and tolerance to oral iron (Bayoumeu *et al.,* 2005; Khalafallah and Dennis, 2012; Neeru *et al.,* 2012). Parenteral iron administration shunts the natural mechanism of intestinal iron absorption and the associated protein binding which allows free and non-protein-bound iron to circulate. Parenteral iron should not be administered if the patient's iron status is not known in order to prevent possible iron overload and also due to the toxic nature of free iron, which enhances the formation of oxygen and hydroxide radicals that can lead to cell and tissue damage (Breymann, 2013). The parenteral iron preparations commonly used in pregnancy include; iron carboxymaltose, iron sucrose, iron sorbitol, iron gluconate (sodium ferric gluconate), iron dextran (high and low molecular weight) and iron polymaltose (iron dextrin) (Breymann, 2013). Iron sucrose and iron carboxymaltose preparations are higher in single dose administrations with short application time (5-15 minutes) and therefore have advantages over iron polymaltose and iron gluconates (Christoph *et al.,* 2012).

Several studies indicated that using parenteral iron for the treatment of iron deficiency anaemia in pregnancy shows more advantageous outcome than oral iron in terms of haemoglobin and serum ferritin levels (Breymann *et al.,* 2011; Froessler *et al.,* 2013; Ortiz *et al.,* 2011). In a study conducted by Khalafallah *et al.* (2012), there was a positive influence of intravenous iron polymaltose during pregnancy on quality of life parameters, postpartum period and a progressing positive effect on maternal iron stores. Certain conditions such as pre-existing anaemia (moderate to severe), side effects of oral iron, refusal of blood transfusion by patient and limited time until delivery or planned operation can influence the use of intravenous iron as the first option in anaemia treatment during pregnancy (Breymann, 2013). There is growing evidence that intravenous iron is more effective as it improves iron stores, offers more rapid haemoglobin correction, and well tolerated than oral iron in treating iron deficiency anaemia during pregnancy.

2.4 Lipid Metabolism in Pregnancy

Normal alterations occur in lipid metabolism of healthy pregnant women which are essential for foetal growth and development. Normal pregnancy has been linked with projected changes in lipid metabolism and elevations in lipid concentration as pregnancy progresses (Hadden and McLauglin, 2009; Vrijkotte *et al.,* 2012). The alterations in lipid metabolism assist in adaption for both the mother and foetus (Pusukuru *et al.,* 2016). The most important features of lipid metabolism alterations during pregnancy are fat accumulation, increased tissue lipolysis and maternal hyperlipidaemia (Grimes and Wild, 2000; Herrera, 2002). These alterations are essential and correspond to clinical manifestations which result in constant elevation of maternal lipid levels from preconception to third trimester (Grimes and Wild, 2000; Jin *et al.,* 2016; Loke *et al.,* 1991). Alterations in serum lipid concentrations during pregnancy are assumed to be affected by hormonal changes such as elevations in concentrations of serum oestrogen and progesterone (Desoye *et al.,* 1987; Kalkhoff, 1982; Pusukuru *et al.,* 2016). Similarly, levels of serum lipid and its metabolism are

significantly affected by hyperinsulinaemia and insulin resistance during pregnancy (Pusukuru *et al.,* 2016).

Pregnancy has also been linked with changes in the composition and size of low density lipoproteins (Mukherjee, 2014). Low density lipoprotein size reduces as triacylglycerol concentration increases with an elevated fraction of smaller and denser low density lipoprotein particles which are supposed to be more atherogenic (Belo *et al.,* 2002; Brizzi *et al.,* 1999). Levels of apolipoprotein A-I and high density lipoprotein are as well elevated during normal pregnancy, with highest levels in the second trimester (Mukherjee, 2014). There are elevations in the levels of apolipoproteins A-I, A-II, and B across gestation, whereas levels of high density lipoprotein initially increase and then drop in the third trimester (Brizzi *et al.,* 1999). In the first trimester, there is significant deposition and hypertrophy of maternal adipocytes with increased expression of insulin receptors in which glucose is available to meet the metabolic demand of the growing foetus (Herrera, 2002). Production of progesterone and high levels of maternal insulin during pregnancy leads to increased synthesis of lipids with reduced lipolysis (Vrijkotte *et al.,* 2012). The lipids are subsequently transported through the placenta and metabolized which indicate the vital role of lipids in normal foetal development (Hadden and McLauglin, 2009).

Furthermore, Wang *et al.* (2018) showed that serum levels of cholesterol, triacylglycerols, low density lipoprotein and high density lipoprotein were significantly elevated from early to mid-pregnancy, with elevation of serum triacylglycerols being the most noticeable features. The intense increase observed in triacylglycerols is caused by reduced activity of lipoprotein lipase and enhanced activity of hepatic lipase resulting in elevated synthesis of triacylglycerol in the liver and its reduced breakdown in adipose tissue (Kershaw and Flier, 2004). The high levels of triacylglycerol are subsequently utilised for maternal metabolic needs, sparing glucose for the foetus (Pusukuru *et al.,* 2016). Other studies also reported similar alterations in maternal lipid concentrations during

pregnancy (Vrijkotte *et al.,* 2012; Zheng *et al.,* 2018). The increase in levels of serum lipids during pregnancy plays vital role in pregnancy progression and foetus development because studies have shown that lipid concentrations fall back to pre-pregnancy levels after delivery (An-Na*et al.,* 1995; Bartels *et al.,* 2012).

2.5 Consequences of Dyslipidaemia in Pregnancy

Pregnancy is a distinctive opportunity for the estimation of subclinical derangements of blood lipids. Mukherjee, (2014) concluded that presence of atherogenic dyslipidemia described as high triacylglycerol, small dense low density lipoprotein and reduced high density lipoprotein levels in early gestation informs a high risk of adverse pregnancy outcomes likewise risk for cardiovascular disease. Atherogenic lipid profile at first trimester of pregnancy has been linked with increased risk of negative pregnancy outcomes such as preterm delivery, maternal morbidity and mortality (Vrijkotte *et al.,* 2012). Singh *et al.* (2018) reported a strong association between dyslipidemia and development of hypertensive disorders of pregnancy as well as intra uterine growth restriction and death of foetus.

Elevated concentrations of maternal cholesterol and triacylglycerol are linked with gestational hypertension (Jan *et al.,* 2012), preterm birth (Catov *et al.,* 2007; Magnussen *et al.,* 2011), preeclampsia (Kandimalla *et al.,* 2011; Ray *et al.,* 2006) and babies large for their gestational age (Kushtagi and Arvapally, 2009). Contrariwise, lower levels of cholesterol in pregnancy are reported to be associated with preterm birth (Edison *et al.,* 2007) with higher risk of the infants to be delivered small for their gestational age (Catov *et al.,* 2010; Sattar *et al.,* 1999). Vrijkotte *et al.* (2012) and Wiznitzer *et al.* (2009) found that unusual elevated concentrations of maternal triacylglycerol in early pregnancy are a major contributor in the manifestation of preeclampsia, pregnancy induced hypertension, induced preterm birth, and children large for their gestational age. There is high risk

for cardiovascular disease later in life for preterm newborns that are small or large for their gestational age including women who gave birth to them (Fanshawe and Ibrahim, 2013; Gluckman *et al.*, 2008).

Furthermore, Catov *et al.* (2010) observed that there was a U-shaped relationship between pre-pregnancy lipid concentrations and the risk of preterm birth in healthy women, likewise pregnant women with abnormal pre-pregnancy cholesterol levels had greater incidence of preterm birth within 34 to 37 weeks of pregnancy. In addition, there exists difference in the pathways of abnormal concentration of cholesterol associated with adverse pregnancy outcomes. Elevation of cholesterol concentration shows a proatherogenic phenotype with greater risk of preterm birth as well as higher cardiovascular risk later in life whereas low levels of cholesterol especially in early pregnancy is linked with increase in triacylglycerol concentrations and accompanied together with higher incidence of foetal intrauterine growth retardation as well as preterm birth (Catov *et al.*, 2010; Sattar *et al.*, 1999).

Alterations in maternal hormones has been associated with the levels of cholesterol, high density lipoprotein, low density lipoprotein and triacylglycerol which subsequently lead to changes in the process of foetal development and pregnancy problems (Corton *et al.*, 2014; Vrijkotte *et al.*, 2011). Wild *et al.* (2015) stated that exposure of the foetus to elevated cholesterol levels and oxidative metabolites of cholesterol has been shown to cause programming of arterial cells with a predisposition to atherosclerosis later in life (Napoli *et al.*, 1997); such findings have been detected in pregnant women that are obese, diabetic and have metabolic syndrome (Palinski and Napoli, 2008). Pregnant women with reduced levels of plasma cholesterol were found to have smaller newborns and microcephaly (Edison *et al.*, 2007). High levels of blood triacylglycerol increase the risk of pancreatitis and clinical features of its severity which are abdominal pain, eruptive xanthoma, dyspnea, hepatosplenomegaly, peripheral neuropathy, dementia and memory loss (Wild *et al.*, 2015)

which negatively affect pregnancy. Dyslipidaemia is highly prevalent globally in all populations (including pregnant women) ranging from 21.7 – 87.7% (Al-Duais and Al-Awthan, 2019; Camacho *et al.,* 2019; Feitosa *et al.,* 2017; Mushtaq *et al.,* 2020) and 11.9 – 45.7% in Africa (Nzeale, 2019). In Nigeria, prevalence of dyslipidaemia was reported to be 71.1% (Onyemelukwe *et al.,* 2017) while in Kano State; the prevalence was found to be approximately 40% (Muhammad *et al.*,2013;Saidu *et al.,* 2015).

2.6 Dietary Pattern and Pregnancy

Dietary pattern is the general profile of food and nutrient consumption which is characterized on the basis of the usual eating habits. The analysis of dietary patterns gives a more comprehensive impression of the food consumption habits within a population. It may be better at predicting the risk of diseases than the analysis of isolated nutrients or foods because the joint effect of various nutrients involved would be better identified (Hu, 2002). Patterns of nutritional behaviors adopted in childhood and adolescents are mostly continued in adult life and increase the risk of development of many chronic diseases (Kapka- Skrzypczak *et al.,* 2012). No one can deny the importance of nutrition in any stages of life, especially during pregnancy. It is essential for the pregnant diet sufficient energy and nutrients to maintain maternal health, to allow for adequate foetal growth and development. According to fetal origin's hypothesis, maternal nutrition during pregnancy has long-term effects on foetus health (Grieger *et al.,* 2014). Factors that lead to a healthy pregnancy include overall health, appropriate weight gain, physical activity during pregnancy, intake of various foods, mineral supplements and vitamins (Procter and Campbell, 2014).

Maternal nutrition is the main determinant of fetal development, birth weight and disease of the infant, as well as the women's health and reproductive capacity. Dietary pattern approach provides more comprehensive knowledge about nutrition and disease (Tucker, 2010). However, a dietary

pattern is population-specific and can be influenced by socio-cultural factors (Volgyi *et al.,* 2013) and food availability (Englund-Ogge *et al.,* 2014; Mishra *et al.,* 2010). Recently, nutritional epidemiologists have focused on dietary patterns, rather than specific macro- or micronutrients, to examine the effects of the diet on health (Starling *et al.,* 2017). Dietary patterns have the benefit of examining foods consumed in the context of the overall diet, allowing for interactive effects between dietary components and accommodating the high correlations that may occur between specific foods or nutrients (Hu, 2002).

A dietary pattern containing several protein-rich food sources, fruit, and some whole grains is associated with reduced likelihood for preterm delivery, whereas a dietary pattern mainly consisting of discretionary items is associated with preterm delivery, shorter birth length, and earlier gestation (Grieger *et al.,* 2014). Poor dietary behaviors in the peri-conceptional period could be altered to promote behavior change in dietary intake to improve perinatal outcomes and the long-term health of the child.

2.7 Relationship between Iron Deficiency Anaemia, Lipid Metabolism and Dietary Pattern in Pregnancy

Currently, there is no available information or report on the relationship between iron deficiency anaemia and lipid profile among pregnant women. Lipid metabolism is principally influenced by iron deficiency because iron serves as a vital cofactor in several key enzymes (such as 3-hydroxy-3-methyl glutarate-CoA reductase, lecithin cholesterol acyltransferase, lanosterol-14α-demethylase and carnitine acyl transferase) involved in metabolism of lipids. Various studies probing on how lipid metabolism is affected in conditions of iron deficiency have generated inconsistent outcomes (Ozdemir *et al.,* 2007). Studies that determined the association of iron deficiency anaemia and lipid metabolism have shown lipid indices to be either high or low in presence of iron deficiency anaemia, however in the latter; the indices were raised upon iron supplementation (Choi *et al.,* 2001; Ozdemir

et al., 2007). Similarly, Vari *et al.* (2007) reported significant positive correlation between iron stores and lipid profile. The findings of Sherman(1979) and Tanzer *et al.*(2001) reported that levels of lipid increases in iron deficiency while lower amount of lipids were recorded by Choi*et al.*(2001)and Ohira *et al.*(1980).

In a large cross-sectional study among Chinese adults, high levels of serum ferritin were associated with the prevalence of dyslipidemia and a significant positive association between serum ferritin levels and lipid parameters were reported (Li *et al.,* 2017). Similarly, Zaribaf *et al.* (2014) reported that there was significant positive correlation between serum ferritin concentration with total cholesterol and triacylglycerol levels among women of reproductive age. Ozdemir *et al.* (2007) showed that triacylglycerol, total cholesterol and low density lipoprotein are reduced in premenopausal women with iron deficiency anaemia. Similarly, reduced amounts of total cholesterol, triacylglycerol, high density lipoprotein, low density lipoprotein and very low density lipoprotein were detected in patients with anaemia compared with normal controls (Sandeep *et al.,* 2014). Sandeep *et al.* (2014) found that concentration of serum cholesterol and triacylglycerol were significantly decreased in severely anaemic patients; however the low serum lipid levels were restored to normal after supplementation with iron. Additionally, it was confirmed that all the sub fractions of lipid profile were considerably low in anaemic cases in comparison with controls where the quantitative decrease was directly correlated with the severity of anaemia (Narra *et al.,* 2005).

In a study conducted among non-obese individuals, those with anaemia showed elevated lipid profile in comparison with non-anaemic controls (Aboromia *et al.,* 2019) and this corroborates with the findingsof Nandyala *et al.* (2013) and Verma *et al.* (2010) which indicatedan association between iron deficiency anaemia and derangements of serum lipids among Indian adults. There was significant reduction in low density lipoprotein in patients with anaemia when compared with healthy adults (Verma *et al.,* 2010). Similarly, Antappanavar *et al.* (2014) demonstrated that

concentration of triacylglycerol and very low density lipoprotein levels were elevated in patients with iron deficiency anaemia compared with non-anaemic adults; contrariwise low density lipoprotein levels were reduced in anaemic patients. In addition, there was elevation in the levels of all lipoproteins (except high density lipoprotein) and triacylglycerol in women with iron deficiency anaemia (Yang *et al.,* 2015). Miri-Aliabad *et al.* (2017) stated that disturbance in carnitine metabolism and reduced levels of serum-free carnitine were the reason for higher amount of serum lipids.

Moreover, iron deficiency was reported to be a risk factor for the development of coronary artery disease as well as linked with the progression of an atherogenic lipid profile and increased oxidative stress (Toescu *et al.,* 2002). Reduced serum iron-binding capacity and elevated serum iron concentrations following derangement in levels of blood lipids have been reported to serve as risk factors for myocardial infarction and coronary artery disease (Magnusson *et al.*, 1994; Salonen *et al.*, 1992). Chopra *et al.* (2015) revealed that there was uninterrupted association between levels of serum iron and ferritin with risk factors of coronary artery disease. It was proposed that iron plays a crucial role in the pathogenesis of atherosclerosis, mainly by serving as a catalyst for the atherogenic modification of low density lipoprotein (Minqin *et al.,* 2005; Yuan and Li, 2003). The progression of an atherosclerotic plaque can be slowed down by lowering body iron stores with phlebotomy (Qayyum and Schulman, 2005; Zheng *et al.,* 2005). Finally, researches using numerous model organisms as well as data from clinical studies have shown links between iron and lipid metabolic pathways, nevertheless the mechanisms involved in these interactions and their role in the progression of human diseases remains unravelled (Rockfield *et al.,* 2018).

Furthermore, the effects of dietary pattern and maternal nutrition on some complications such as gestational diabetes mellitus, pre-eclampsia, intrauterine growth limitation, low birth weight and small for gestational age has been reported in various studies (Kalhor *et al.,* 2015; Ramakrishnan *et*

al., 2012). Several contributing factors including low physical activity, smoking and unhealthy diet practices have been linked to dyslipidaemia and subsequently cardiovascular diseases, worldwide (Esmaillzadeh *et al.,* 2007).

Iron deficiency anaemia, abnormal lipid levels and poor dietary pattern have all been reported to be linked to negative pregnancy outcomes such as preterm birth, low birth weight, babies small-for-gestational age, gestational diabetes mellitus, hypertension and pre-eclampsia (Kalhor *et al.,* 2015; Milman, 2011; Singh *et al.,* 2018). It is therefore obvious that dietary pattern (habits and frequency of food consumption), iron status and lipid metabolism are associated. Dietary pattern couldhave effect on the iron status because the more the frequency of consumption of iron-rich foods, the more the iron levels in the body; although bioavailability, intestinal absorption and body regulation/homeostasis are factors that could affect the association. Similarly, consumption pattern of lipid-rich foods could affect the levels of lipids; abnormal lipid levels have been associated with dyslipidaemia and risk for cardiovascular diseases (Mukherjee, 2014). Diets consumed (which can be affected by dietary practices) are major components of several molecules such as enzymes and hormones which are involved in the metabolism and regulation of both iron and lipids in the body system, hence dietary pattern could affect iron and lipid levels especially during pregnancy when there is rapid alterations in the levels and requirements of iron and lipids.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

Validated semi-structured questionnaire, Plain and EDTA (Ethylene Diamine Tetraacetic Acid) tubes, Water-bath(Thermo Scientific, TSGP28), Micropipette, Cuvette, Centrifuge (MPW-223e), Spectrophotometer (20D Techmel & Techmel USA), Microtitre plate and reader (Wincom DNM-9602)

3.1.1 Chemicals and reagents

All chemicals, reagents and assay kits were of analytical grade and purchased from reputable companies.

3.2 Methods

3.2.1 Study area

This study was carried out at Aminu Kano Teaching Hospital (AKTH), Kano, Nigeria. AKTH is a government tertiary teaching hospital located at 11.9634° N, 8.5504° E, Unguwa Uku, Zaria Road, Kano State, Nigeria. The core function of the teaching hospital is service delivery, teaching and research. Antenatal and delivery services to pregnant women and other services related to general healthcare are provided in the Obstetrics and Gynaecology Department of the hospital. Antenatal clinics are run from Monday to Thursday starting at 7:00am.

3.2.2 Ethical clearance

This study was performed in line with the principles of the Declaration of Helsinki. Ethical clearance for the study was obtained from the institutional ethical committees of AKTH, Kano (Appendix

1a)and Ahmadu Bello University (ABU), Zaria (Appendix 1b) as well as Health Research Ethics committee, Ministry of Health, Kano state (Appendix 1c). Informed written and/or verbal consent was obtained from each subject before inclusion into the study (Appendix 2).

3.2.3 Study design

The study waslongitudinally designed in order to evaluate the changes in iron deficiency anaemia, serum lipid profile and dietary pattern among pregnant women attending antenatal care unit AKTH, Kano, Nigeria. Blood samples were collected from consented pregnant women in their second trimester upon confirmation from theultrasound scanning results. The pregnant women wererequested to revisit the antenatal unitafter 12 weeks whichsupposedly would be in the third trimester.Upon the revisit,the blood samples were collected again. The flowchart of the study is shown in Figure 3.1.

3.2.4 Study participants

All pregnant women within the age of 15-49 years in their second trimester attending the antenatal care unit, AKTH at the time of study were recruited.

3.2.4.1 *Exclusion criteria*

i. Pregnant women with medical complications such as diabetes mellitus, hypertension, cardiovascular diseases, HIV/AIDS, cancer and haemoglobinopathies were excluded.
ii. Pregnant women who refuse to give consent were also excluded.
iii. Pregnant women below 15 years and above 49 years were excluded.

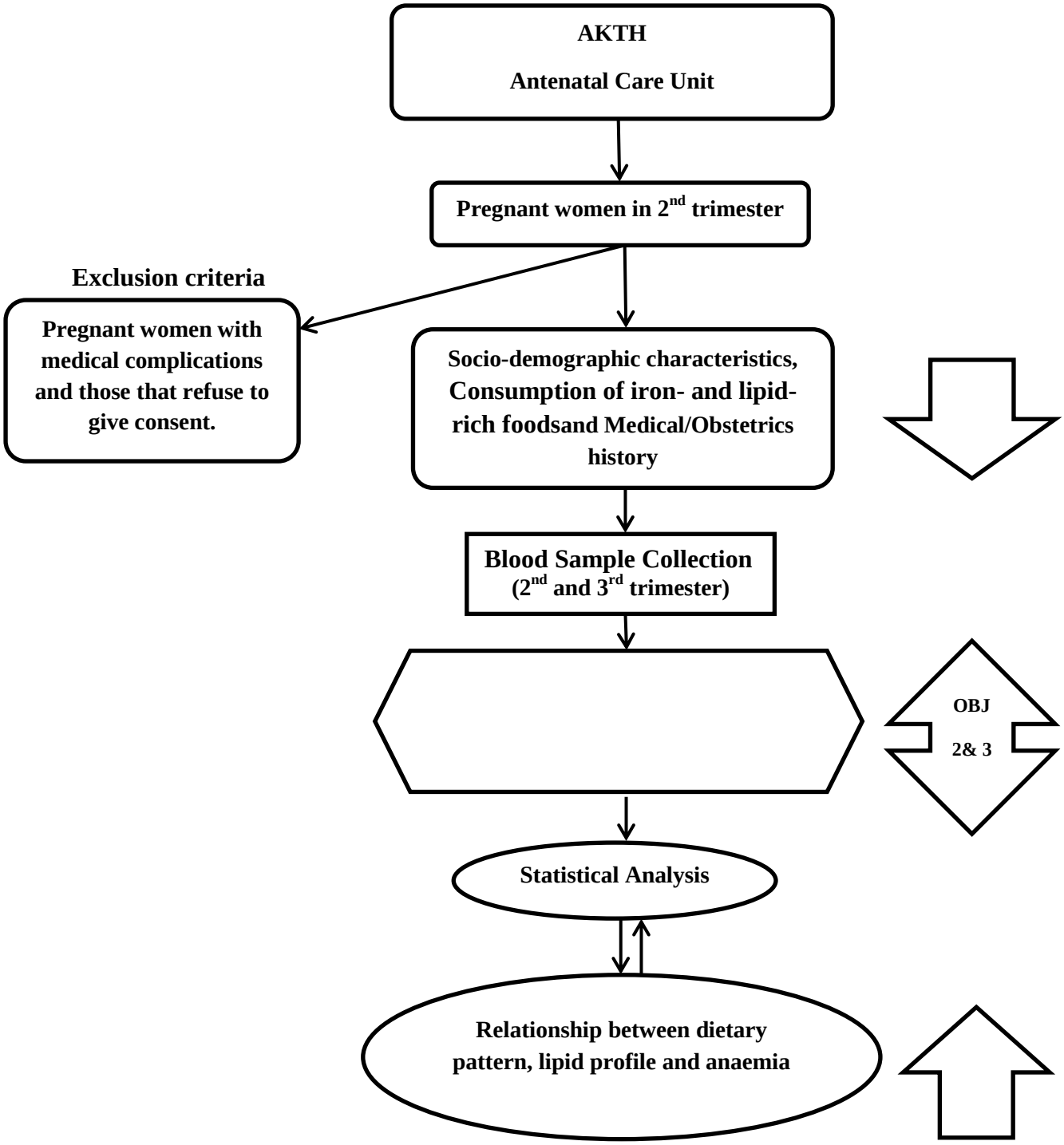


Figure 3.1: Flowchart of the study on association between dietary pattern, lipid profile and anaemia among pregnant women attending antenatal care unit, AKTH, Kano, Nigeria.

3.2.5 Sample size

The sample size was calculated using the Dobson's formula:

$$n = t^2 \frac{p \times q}{d^2}$$

Where n= Sample size,

t= Confidence interval at 95% (1.96),

p= Prevalence of anaemia among pregnant women in first trimester (6.50%) as reported by Nwizu *et al.* (2011)

q= 1− p (1− 0.065= 0.935),

d= Desired level of significance (0.05)

Substituting and adjusting for attrition rate at 20% and putting design effect of 1.0

Sample size (n) = 113

3.2.6 Sampling technique

Simple random sampling was used for selection; targeting pregnant women in second trimester visiting the antenatal care unit, AKTH, Kano. Approximately, 6 pregnant women were selected daily and randomly using table of random numbers for 5 weeks and a total of 112 pregnant women were recruited. Hence,the study started with an initial number of one hundred and twelve (112) pregnant women in their second trimester attending antenatal care unit, AKTH, however only eighty-five (85) pregnant women were completely followed up to third trimester. The drop out was due to reasons ranging from premature labour, complications and withdrawal from the study although a 20% attrition rate was included in the sample size calculation to account for such instances.

3.2.7 Data collection

3.2.7.1 *Demographic, dietary and medical/obstetrics data collection*

A validated semi-structured questionnaire containing three sections was administered to consented pregnant women by trained interviewers so as to collect data on socio-demographic characteristics, consumption of iron- and lipid-rich foods and medical/obstetrics history (Appendix 3).

3.2.7.2 *Blood sample collection*

Five millilitres of blood sample was collected by a trained phlebotomist using venipuncture; immediately 1 ml was transferred into an EDTA tube and 4 ml into a plain tube. The blood in plain tube was allowed to clot and then centrifuged to obtain serum and preserved at -20^{o}C for further analyses. Precautions taken include; appropriate use of a needle-a gauge for the vessel; gentle pressing of the syringe plunger in order not to force the blood into the tube; thus decreasing the shear force on the red blood cells, avoid drawing blood specimens from an intravenous or central line, avoid under-filling a tube so that the ratio of anticoagulant to blood is greater than 1:9, avoid mixing of tube too vigorously, use of gloves and disinfecting the area of puncture as well as allowing the alcohol or disinfectant to dry (WHO, 2010).

3.2.7.3 *Determination of haemoglobin concentration*

Blood sample in the EDTA tubewas used for estimation of haemoglobin concentration. Cyanmethaemoglobin method was used as described by NCCLS (2000) for the determination using freshly prepared Drabkin's solution. In solution, ferrous ions of haemoglobin are oxidized to the ferric state by potassium ferricyanide to form haemiglobin which in turns reacts with cyanide ions provided by potassium cyanide and forms haemiglobin cyanide. Twenty microlitre of blood was added to 5 ml of Drabkin's solution made up of potassium cyanide (50mg), potassium ferricyanide (200mg), potassium dihydrogen phosphate (140mg), Triton-X 100 (1ml), dissolved in 1 L of distilled water and the absorbance was measured at 540nm after 5 minutes of incubation at room temperature(25^{o}C) (NCCLS,2000). Haemoglobin concentration was calculated as follows:

$$Haemoglobin\ Conc.(g/dl) = \frac{Absorbance\ of\ Sample}{Absorbance\ of\ Standard} \times Concentration\ of\ Standard$$

3.2.7.4 *Determination of serum iron, total iron binding capacity (TIBC) and transferrin saturation*

Serum iron and TIBC were determined using commercial Centronic GmbH assay kits (Am Kleinfeld 11, 85456 Wartenberg/Germany) following the manufacturer's instructions. Serum ironwas determined according to the method described by Makino *et al.* (1988) where transferrin-bound iron was released at an acidic pH and reduced from ferric to ferrous ions. These ions react with ferrozine to form a violet coloured complex. Fifty microlitreeach of sample, standard and distilledwater was added to 1000µl of reagent (Guanidine hydrochloride pH 4.0-4.4) in separate tubes; the contents were mixed and incubated at 25^{o}C for 10 minutes. The absorbance of the mixtures was measured at 560nm which was proportional to serum iron concentration. Iron concentration was calculated as follows:

$$Iron\ Conc.(\mu mol/L) = \frac{Absorbance\ of\ Sample}{Absorbance\ of\ Standard} \times Concentration\ of\ Standard$$

Total iron binding capacity was estimated as described by Stookey (1970) and Yamanishi *et al.* (2003). Serum was treated with excess of ferrous ions to saturate the iron binding sites on transferrin. The excess ferrous ions was adsorbed and precipitated and the iron content in the supernatant was measured to give the TIBC. Two hundred and fifty microlitre of sample was added to 500µl of $FeCl_3$, mixed and allowed to stand for 30 minutes at 25^{o}C. One spatula of magnesium carbonate was added to the mixture and kept for 60 minutes; the mixtures were shakenintermittently within the period and then centrifuged for 10 minutes at 4000rpm. The clear supernatant was used for iron measurement as described above by Makino *et al*. (1988).

Transferrin saturation was calculated using the formula as estimated by Stookey (1970):

$$Transferrin\ saturation\ (\%) = \frac{Serum\ Iron\ Concentration}{TIBC} \times 100$$

3.2.7.5 *Determination of serum ferritin*

Serum ferritin was determined using Human Ferritin Enzyme Immunoassay Test Kit following manufacturer's protocol. The Ferritin Quantitative Test Kit was based on a microplate immunoenzymometricassay (Addison *et al.,* 1972; Beamish *et al.,* 1974). The assay system utilized one anti-ferritin antibody for solid phase (microtitre wells) immobilization and another mouse monoclonal anti-ferritin antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample was allowed to react simultaneously with the antibodies, resulting in the ferritin molecules being sandwiched between the solid phase and enzyme-linked antibodies. The microplate wells were formatted and 25µl each of serum reference, control and sample was added to the respective well, 100µl of biotinylated monoclonal mouse IgG was added into each well, the microplate was swirled gently for 30 seconds and covered, it was then incubated for 30 minutes at room temperature (25^{o}C). The contents of the microplate were decantated and then 350µl of wash buffer (surfactant in buffered saline) was added, followed by decantation; the washing was done two more times. After washing, 100µl of horseradish peroxidase was added to each well and the plate was incubated for 30 minutes at room temperature (25^{o}C). The contents were again discarded by decantation and washed three times as described earlier. Thereafter, 100µl of working substrate solution (3, 3', 5, 5'-tetramethlybenzidine and hydrogen peroxide) was added to all wells and incubated at room temperature (25^{o}C) for 15 minutes after which 50µl of stop solution (1N HCl) was added to each well and mixed gently for 20 seconds. The absorbance in entire wells was read at 450nm in a microplate reader. Concentration of serum ferritin was extrapolated from a ferritin standard curve (Appendix 4).

3.2.7.6 *Determination of lipid profile and atherogenic index*

Total cholesterol, triacylglycerol and high density lipoprotein (HDL) were determined in the serum using commercial Agappe assay kits following the manufacturer's instructions. Low density lipoprotein (LDL), very low density lipoprotein (VLDL) and atherogenic index were calculated.

The total cholesterol was determined as described by Allain *et al.* (1974).Total cholesterol was estimated after enzymatic hydrolysis and oxidation where the indicator quinoneimine was formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase.Tenmicrolitreeach of sample, standard and distilled water was added to 1000µl of reagent (cholesterol esterase and oxidase, peroxidase, 4-aminoantipyrine and phenol) in separate tubes; they were mixed and incubated at 37^{o}C for 5 minutes. The absorbance of the mixtures was measured at 546nm which was proportional to totalcholesterol concentration. Cholesterol concentration was calculated as follows:

$$Cholesterol\ Conc.(mg/dl) = \frac{Absorbance\ of\ Sample}{Absorbance\ of\ Standard} \times Concentration\ of\ Standard$$

The triacylglycerol was determined as described by Jacobs and VanDemark (1960). It involves enzymatic hydrolysis with lipases and the indicator was a violet coloured complex formed from hydrogen peroxide, 4-aminoantipyrine and N-ethyl-N-(3-sulfopropyl)-3-methylaniline (TOPS) sodium salt monohydrateunder the catalytic influence of peroxidase. Tenmicrolitreeach of sample, standard and distilledwater was added to 1000µl of reagent (lipoprotein lipase, glycerol kinase, glycerol-3-phosphate oxidase, peroxidase, 4-aminoantipyrine and TOPS) in separate tubes; they were mixed and incubated at 37^{o}C for 5 minutes. The absorbance of the mixtures was measured at 546nm which was proportional to triacylglycerol concentration.Triacylglycerol concentration was calculated as follows:

$$Triacylglycerol\ Conc.(mg/dl) = \frac{Absorbance\ of\ Sample}{Absorbance\ of\ Standard} \times Concentration\ of\ Standard$$

High density lipoprotein was estimated through quantitative precipitation of low and very low density lipoproteins and chylomicron fractions by addition of phosphotungstic acid in the presence of magnesium ions as described by Assman (1979).Three hundred microlitre of sample was added to 300µl of HDL reagent (phosphotungstate and magnesium chloride), it was mixed and allowed to stand for 10 minutes at room temperature (25^{o}C) which was followed by centrifugation for 10 minutes at 4000rpm. After centrifugation, the clear supernatant from the precipitate was used for determination of HDL cholesterol concentration as described by Allain *et al*. (1974) using 50µl of HDL standard as the reference. Concentration of HDL cholesterol was calculated as follows:

$$HDL\ Cholesterol\ Conc.(mg/dl) = \frac{Absorbance\ of\ Sample}{Absorbance\ of\ Standard} \times Concentration\ of\ Standard\ \times 2$$

Low density lipoprotein was calculated using Friedwald's formula (Friedwald *et al.,* 1972):

$$Low\ density\ lipoprotein = Total\ cholesterol - HDL - \frac{Triacyglycerol}{5}$$

Very low density lipoprotein was derived using the formula as determined by Crook (2006):

$$Very\ low\ density\ lipoprotein = \frac{Triacylglycerol}{2.2}$$

Atherogenic index was derived using the formulaas estimated by Dobiasova and Frohlich (2001):

$$Atherogenic\ index = Log\ \frac{Triacylglycerol}{HDL}$$

3.2.7.7 *Determination of dyslipidaemia*

Dyslipidemia was determined according to the guidelines set by National Cholesterol Education Program-Adult Treatment Panel (NCEP-ATP III) (NCEP-ATP, 2001). Total cholesterol, triacylglycerol and LDL values were considered above normal levels if ≥200 mg/dl, ≥150 mg/dl and

≥130 mg/dl respectively while HDL values <50 mg/dl was considered below normal level. Dyslipidaemia was recorded when at least one of above parameters was affected.

3.2.7.8 *Calculation of prevalence of anaemia, iron deficiencyand iron deficiency anaemia*

The prevalences of anaemia and iron deficiency were based on haemoglobin level of <11.0 g/dl (WHO, 2001) and serum iron concentration of <8.0 µmol/L (Abbassi-Ghanavati *et al.,* 2009) respectively, while that of iron deficiency anaemia was based on transferrin saturation of <14.0% (Abbassi-Ghanavati *et al.,* 2009) and serum ferritin concentration of <12 ng/ml(National Academy of Sciences Panel, 1990). The prevalence of each status based on the respective biochemical indicator was calculated as follows:

$$Prevalence = \frac{Number\ of\ sample\ with\ conc. < normal\ range\ of\ each\ indicator}{Total\ number\ of\ sample} \times 100$$

3.2.8 Statistical analysis

Data collected werestored, managed and analyzed using Statistical Package for Social Sciences (SPSS) IBM software version 21 and Microsoft Excel 2010. Analysis of each assay was done in triplicate and data were represented as proportion (percentage) or mean ± standard deviation as appropriate. Descriptive statistics were performed to summarize demographic, consumption pattern and medical/obstetrics data. Normality and Levene's test were conducted. Paired sample t-test/Wilcoxon Signed Rank test were conducted for comparison of data while Pearson/Spearman correlation and *Eta* test were conducted to determine relationship and association respectively. P-value ≤0.05 was considered statistically significant. Access to research data was restricted to the investigator and their supervisor. All opinions and data were reported in aggregated form in such a way that individual persons are not identifiable. All written materials (questionnaires) and electronic

information were kept in a locked file and in a password protected file respectively and access restricted to the investigator and supervisor.

CHAPTER FOUR

4.0 RESULTS

4.1 Socio-demographic Characteristicsand Medical/Obstetrics History of Pregnant Women Attending Antenatal Care Unit, AKTH, Kano

The socio-demographic characteristics revealed that majority (49.1%, 55)of the pregnant women were within the age range of 19-28 years, with 67(59.9%) having ≤ 5 people in their household and 65 (58.0%) had tertiary education, while 37 (33.0%) had senior secondary education as indicated in Table 4.1. Among the pregnant women, 101 (90.2%) were married while 7 (6.3%) were divorced/separated; 66 (58.9%) had 1-4 children, while 28 (25.0%) were primiparous. Moreover, majority of the pregnant women (83.0% and 50.0%) were Hausaand full-time housewives respectively, whereas 21 (18.8%) were civil servants (Table 4.1). In terms of household monthly income, 48 (42.9%) earned the sum of₦30,000 – ₦49,999, whereas 45 (40.2%) earned ₦50,000and above (Table 4.1).

Table 4.1: Socio-demographic Characteristics of Pregnant Women Attending Antenatal Care Unit, AKTH, Kano

Socio-demographic Characteristics	Frequency (n=112)	Percentage (%)
Age (years)		
19-28	55	49.1
29-38	48	42.9
39-48	9	8.0
Number of People in Household		
≤ 5	67	59.9
6-10	37	33.0
11-15	6	5.3
> 16	2	1.8
Level of Education		
Non formal	3	2.7
Primary	3	2.7
Junior secondary	4	3.6
Senior secondary	37	33.0
Tertiary	65	58.0
Marital Status		
Married	101	90.2
Divorced/Separated	7	6.3
Widowed	3	2.7
Single	1	0.9
Number of Children		
None	28	25.0
1-4	66	58.9
5-8	18	16.1
Occupation		
Full housewife	56	50.0
Artisan/Skilled worker	14	12.5
Civil servant	21	18.8
Student	13	11.6
Others	8	7.1
Household Monthly Income**		
< ₦10,000	5	4.5
₦10,000 – ₦29,999	14	12.5
₦30,000 – ₦49,999	48	42.9
≥ ₦50,000	45	40.2
Ethnicity		
Hausa	93	83.0
Igbo	5	4.5
Yoruba	4	3.6
Others	10	8.9

**₦1000 is equivalent to 2.63USD

The medical history of the pregnant women showed that 98 (87.5%) have not been diagnosed of any disease as at the time of data collection. Among the pregnant women, 55 (49.1%) were currently on supplement with 31 (56.4%) taking iron/folate supplements and 17 (30.9%) were taking multivitamin as shown in Table 4.2. Supplement prescription to the pregnant women was majorly (76.4%, 42) by medical doctors, however 12 (21.8%) were prescribed by the nurses (Table 4.2). Obstetrics data among the pregnant women indicated that majority (83.0%, 93) were pregnant for more than once, 51 (45.5%) had given birth 2-4 times, 25 (22.3%) had delivered 5 or more times, whereas 13 (11.6%) had given birth only once and 23 (20.5%) had never delivered (Table 4.2). Most of the pregnant women (38.4%, 43) were within gestational age of 19-22 weeks, 46 (41.1%) had experienced miscarriage or abortion in previous pregnancies; conversely 36 (32.1%) of the pregnant women practice 12-24 months of birth spacing while 46 (41.1%) waited for more than 24 months before any pregnancy (Table 4.2).

Table 4.2: Medical/Obstetrics History of Pregnant Women Attending Antenatal Care Unit, AKTH, Kano

Medical/Obstetrics Data	Frequency (n=112)	Percentage (%)
Disease Status		
Yes	14	12.5
No	98	87.5
Disease Type		
None	98	87.5
Ulcer	8	7.1
Asthma	3	2.7
Others	3	2.7
Supplementation Intake		
Yes	55	49.1
No	57	50.9
Type of Supplements*		
Iron/Folate	31	56.4
Multivitamin	17	30.9
Calcium	4	7.3
Others	3	5.4
Supplement Prescriber*		
Doctor	42	76.4
Nurse	12	21.8
Chemist/Shop	1	1.8
Gravidity		
Primigravida	19	17.0
Multigravida	93	83.0
Parity		
Nulliparous	23	20.5
Primiparous	13	11.6
Multiparous	51	45.5
Grand multiparous	25	22.3
Gestational Age (weeks)		
14-18	39	34.8
19-22	43	38.4
23-26	30	26.8
Miscarriage/Abortion		
Yes	46	41.1
No	66	58.9
Birth Spacing		
Not applicable	27	24.1
< 12 months	3	2.7
12 – 24 months	36	32.1
> 24 months	46	41.1

* = Sample size is 55 which comprises of pregnant women taking supplementation.

4.2 Consumption Pattern of Some Iron- and Lipid-rich Foods among Pregnant Women Attending Antenatal Care Unit, AKTH, Kano

The daily, weekly and monthly consumption of haem and non-haem iron sources, enhancers and inhibitors of iron absorption and lipid-rich foods among the pregnant women were presented in Table 4.3 and 4.4. The proportion of pregnant women that consumed red meat, organ meat, chicken and fish (haem-iron sources) at least 1-3 times daily were 34.0%, 13.0%, 14.0% and 38.0% respectively as presented in Table 4.3. However, the results for weekly consumption revealed that 55.0%, 33.0%, 46.0% and 30.0% of the pregnant women consumed red meat, organ meat, chicken and fish respectivelyat least 1-3 times weekly (Table 4.3).

The proportion of pregnant women that consumed egg, beans, soybean, pumpkin, spinach and moringa (non-haem iron sources) in a day at least 1-3 times were 38.0%, 37.0%, 14.0%, 11.0%, 22.0% and 13.0% respectively; while 40.0%, 45.0% and 70.0% of the pregnant women consumed egg, beans and spinach respectively atleast 1-3 times in a week (Table 4.3). In terms of enhancers and inhibitors of iron absorption, pregnant women that consumed orange, lettuce, carrot and sweet potato (enhancers) in a day were 38.0%, 25.0%, 20.0% and 18.0% respectively, while 37.0% of the pregnant women consumed coffee/tea (inhibitor) at least 1-3 times in a day (Table 4.3).

Table 4.3: Consumption Pattern of Some Haem- and Non-haem-iron-rich Foodsand Enhancers/Inhibitors of Iron absorption among Pregnant Women Attending Antenatal Care Unit, AKTH, Kano

Consumption Pattern	**Daily (%)**		**Weekly (%)**		**Monthly (%)**		
Foods	**1-3 times**	**4 times or more**	**1-3 times**	**4 times or more**	**None**	**1-3 times**	**4 times or more**
Haem-Iron-Rich							
Red meat	34	4	55	3	-	4	-
Organ meat	13	1	33	7	11	8	27
Chicken	14	3	46	6	1	10	20
Fish	38	5	30	10	-	13	4
Non-Haem-Iron-Rich							
Egg	38	3	40	19	-	-	-
Beans	37	2	45	16	-	-	-
Soybean	14	3	38	6	-	8	31
Pumpkin	11	-	41	5	14	13	30
Spinach	22	4	70	4	-	-	-
Moringa	13	2	45	8	-	-	32
Enhancers							
Orange	38	7	55	-	-	-	-
Lettuce	25	6	51	18	-	-	-
Carrot	20	5	50	14	-	11	-
Sweet potato	18	3	55	15	-	9	-
Inhibitor							
Coffee/Tea	37	5	50	8	-	-	-

Values are expressed as percentages of frequency of consumption among pregnant women, n = 112.

In addition, the results revealed that the proportion of pregnant women who consumed nuts, vegetable oil, palm oil, milk, butter and sardines (lipid-rich foods) at least 1-3 times in a day were 21.0%, 61.0%, 63.0%, 37.0%, 13.0% and 8.0% respectively, while in terms of weekly consumption, 35.0%, 45.0%, 55.0%, 37.0% and 36.0% of the pregnant women consumed coconuts, milk, yoghurt, butter and sardines at least 1-3 times respectively (Table 4.4).

Table 4.1: Consumption Pattern of Some Lipid-rich Foods among Pregnant Women Attending Antenatal Care Unit, AKTH, Kano

Consumption Pattern	Daily (%)		Weekly (%)		Monthly (%)		
Foods	1-3 times	4 times or more	1-3 times	4 times or more	None	1-3 times	4 times or more
Lipid-Rich							
Avocados	5	-	5	2	68	13	7
Nuts	21	-	57	6	-	16	-
Olive oil	13	-	22	2	35	20	8
Vegetable oil	61	12	27	-	-	-	-
Palm oil	63	8	29	-	-	-	-
Coconuts	21	5	35	9	5	25	-
Milk	37	4	45	14	-	-	-
Yoghurt	13	2	55	12	5	13	-
Butter	13	3	37	13	10	14	10
Sardine	8	1	36	8	21	26	-
Beef tallow	3	1	13	4	61	12	6
Mayonnaise	12	3	26	11	20	8	20

Values are expressed as percentages of frequency of consumption among pregnant women, n = 112.

4.3 Iron Deficiency Anaemia among Pregnant Women Attending Antenatal Care Unit, AKTH, Kano

Results showed that there was significant ($p<0.05$) decrease in the mean values of haemoglobin, serum iron, transferrin saturation and serum ferritin levels from second to third trimester among the pregnant women investigated, whereas level of total iron binding capacity was significantly ($p<0.05$) increased in third trimester compared to the second trimester as presented in Table 4.5.

Table 4.5: Iron Indices of Pregnant Women Attending Antenatal Care Unit, AKTH, Kano

Iron Indices	**Second Trimester**	**Third Trimester**	**p-value**
Haemoglobin (g/dl)	11.8 ± 2.7^{a}	9.9 ± 2.3^{b}	0.01
Serum Iron (µmol/L)*	19.0 ± 6.1^{a}	13.4 ± 5.6^{b}	0.01
Total Iron Binding Capacity (µmol/L)*	81.1 ± 16.7^{a}	114.3 ± 18.5^{b}	0.01
Transferrin Saturation (%)	24.5 ± 9.7^{a}	12.2 ± 6.0^{b}	0.01
Serum Ferritin (ng/ml)	31.7 ± 12.2^{a}	20.6 ± 9.2^{b}	0.01

Values are expressed as Mean ± SD (**n=85**). Values bearing different superscript alphabets for the iron indices across the trimesters are significantly different, "Paired Sample t-test; Wilcoxon Signed Rank test was conducted for iron indices withasterisk (*)".

The prevalence of anaemia (based on haemoglobin level), iron deficiency (based on serum iron level) and iron deficiency anaemia (based on transferrin saturation and serum ferritin levels) among the pregnant women during the second trimester were 37.5%, 8.0%, 10.7% and 14.0% respectively, which significantly ($p<0.05$) increased to 65.8%, 12.9%, 30.6% and 21.2% respectively during the third trimester as indicated in Table 4.6.

Regarding the severity of anaemia, pregnant women in the second trimester with mild, moderate and severe anaemia were 16.0%, 17.9% and 3.6% respectively; whereas 62.5% of the pregnant women were normal in the second trimester which later dropped to 34.2% in the third trimester as shown in Table 4.6. Similarly, the proportion of mildly anaemic pregnant women dropped to 12.9% in the third trimester while those of moderately and severely anaemic pregnant women increased to 40.0% and 12.9% respectivelyduring the third trimester (Table 4.6).

Table 4.6: Prevalence of Anaemia, Iron Deficiency and Iron Deficiency Anaemia among Pregnant Women Attending Antenatal Care Unit, AKTH, Kano

Prevalence	Second Trimester n=112 (%)	Third Trimester n=85 (%)
Non-anaemic	70 (62.5)	29 (34.2)
Anaemia		
Mild (Hb < 11.0)	18 (16.0)	11 (12.9)
Moderate (Hb < 10.0)	20 (17.9)	34 (40.0)
Severe (Hb < 7.0)	4 (3.6)	11 (12.9)
Total	42 (37.5)	56 (65.8)
Iron Deficiency	9 (8.0)	11 (12.9)
Iron Deficiency Anaemia	12 (10.7)	26 (30.6)
	16 (14.0)*	18 (21.2)*

Values with asterisk (*) are prevalence based on serum ferritin. Indices used as criteria: Anaemia based onhaemoglobin < 11.0 g/dl (WHO, 2001); Iron deficiency based on serum iron <8.0 and <5.0 µmol/L (Abbassi-Ghanavati *et al.,* 2009) for second and third trimester respectively; Iron deficiency anaemiabased on transferrin saturation <14.0 and <9.0% (Abbassi-Ghanavati *et al.,* 2009) for second and third trimester respectively and serum ferritin <12 ng/ml (National Academy of Sciences Panel, 1990). WHO classification of severity of anaemia in pregnancy: Non-anaemic = 11.0 g/dl and above; Mild anaemia = 10.0 – 10.9 g/dl; Moderate anaemia = 7.0 – 9.9 g/dl; Severe anaemia = < 7.0 g/dl. Hb = Haemoglobin concentration

4.4 Changes in Lipid Profile,Prevalence of Dyslipidaemia and Atherogenic Index across Trimesters among Pregnant Women Attending Antenatal Care Unit, AKTH, Kano

Lipid profileand atherogenic index among pregnant women attending antenatal care unit in AKTH were presented in Table 4.7. Results indicated that concentrations of cholesterol, triacylglycerol, low density lipoprotein, very low density lipoprotein and atherogenic index were significantly ($p<0.05$) higher in third trimester when compared to second trimester;however,a significant ($p<0.05$) decrease in high density lipoprotein levels were observed in third trimester(Table 4.7).

Table 4.7: Lipid Profile and Atherogenic Index of Pregnant Women Attending Antenatal Care Unit, AKTH, Kano

Lipid Parameters	**Second Trimester**	**Third Trimester**	**p-value**
Cholesterol (mg/dl)*	175.40 ± 39.20^{a}	199.84 ± 37.84^{b}	0.01
Triacylglycerol (mg/dl)	134.29 ± 44.24^{a}	178.71 ± 48.20^{b}	0.01
High Density Lipoprotein (mg/dl)	48.77 ± 11.57^{a}	46.86 ± 11.29^{b}	0.01
Low Density Lipoprotein (mg/dl)*	99.71 ± 43.97^{a}	117.22 ± 41.67^{b}	0.01
Very Low Density Lipoprotein (mg/dl)	61.06 ± 20.13^{a}	81.25 ± 21.90^{b}	0.01
Atherogenic Index*	0.43 ± 0.20^{a}	0.58 ± 0.19^{b}	0.01

Values are expressed as Mean ± SD (**n=85**). Values bearing different superscript alphabets for the lipid parameters across the trimesters are significantly different, "Paired Sample t-test; Wilcoxon Signed Rank test was conducted for lipid parameters with asterisk (*)".

The prevalence and pattern of dyslipidaemia during the second and third trimesters among the pregnant women in AKTH, Kano was presented in Figure 4.1. The prevalence of dyslipidaemia, hypercholesterolaemia, hypertriglyceridaemia, high LDL and low HDL during the second trimester were 69.6%, 19.6%, 36.6%, 18.8% and 49.1% respectively, which subsequently increased to 91.8%, 54.1%, 75.3%, 40.0% and 62.4% respectively during the third trimester as indicated in Figure 4.1.

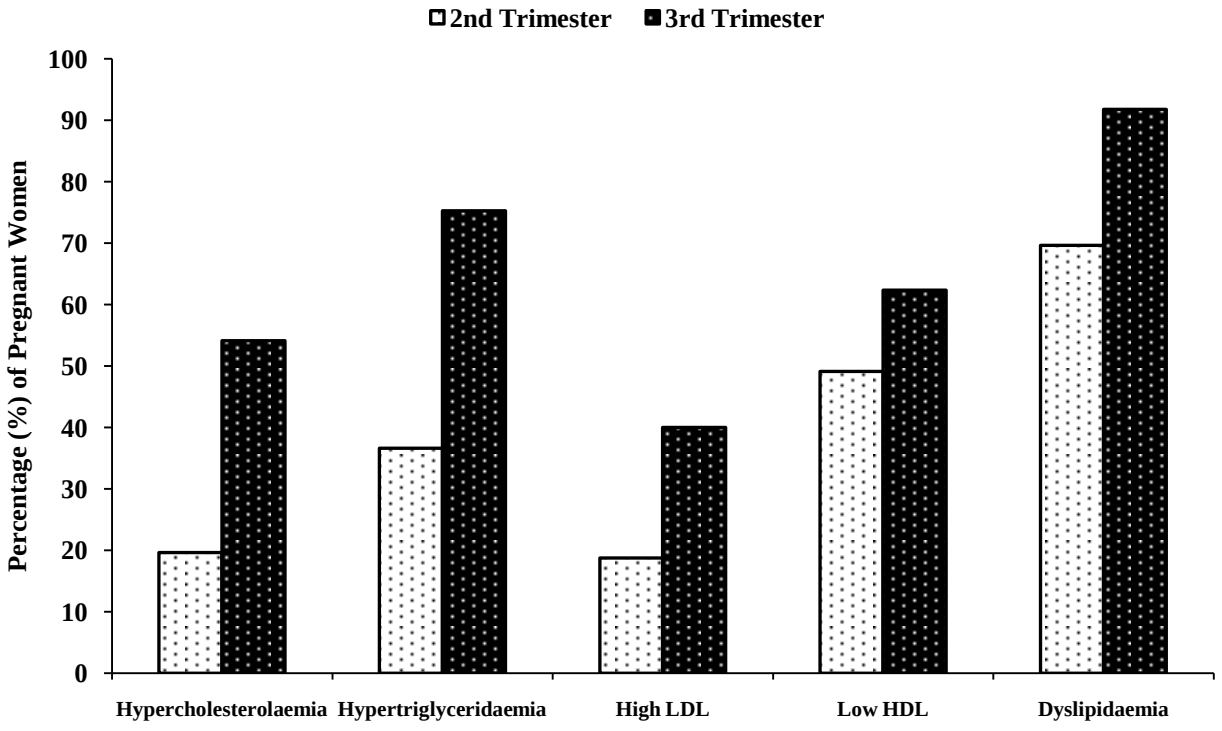


Figure 4.1: Prevalence and Pattern of Dyslipidaemia among Pregnant Women AttendingAntenatal Care Unit AKTH, Kano

Values are expressed as percentages of pregnant women with dyslipidaemia (n=112 and 85 in second and third trimesters respectively). Classification of dyslipidaemia was based on the NCEP-ATP III guidelines (NCEP-ATP, 2001). **HDL** = High density lipoprotein; **LDL** = Low density lipoprotein

Among the pregnant women, 81.0%, 12.0% and 7.0% have high, medium and low risk for cardiovascular diseases respectively in the second trimester whereas proportion of the pregnant women in third trimester with high, medium and low risk for developing cardiovascular diseases were found to be 85.0%, 9.0% and 6.0% respectively as shown in Figure 4.2 using the atherogenic index.

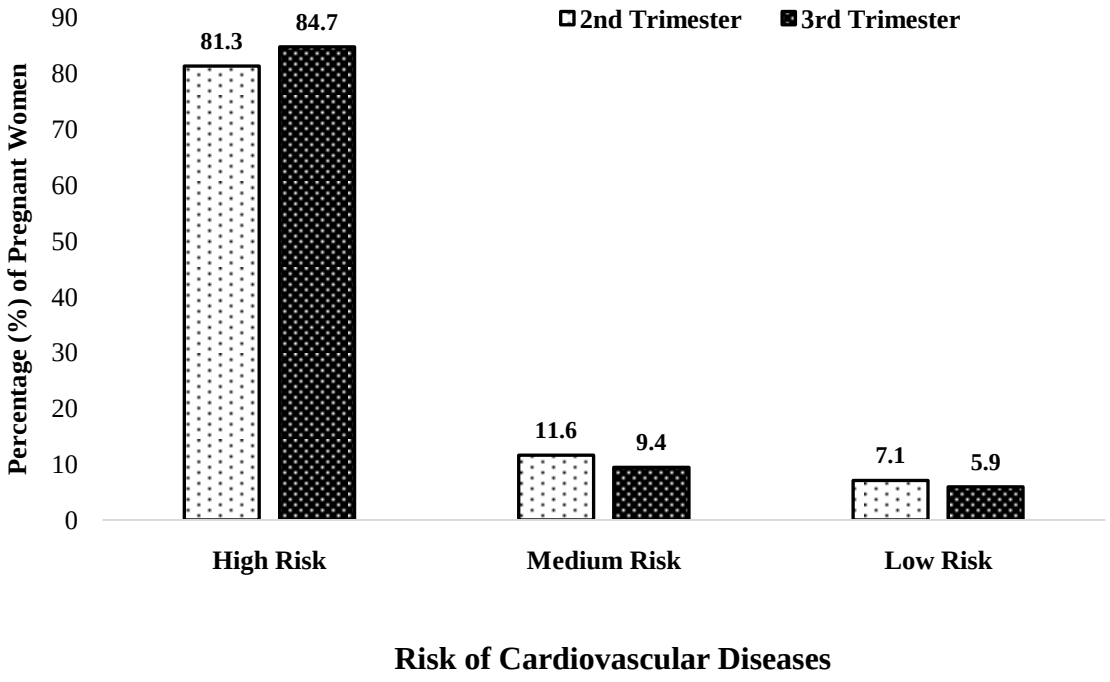


Figure 4.2:Risks for Cardiovascular Diseases among Pregnant Women Attending Antenatal Care Unit, AKTH, Kano

Values are expressed as percentages of pregnant women with risk of cardiovascular diseases (n=112 and 85 in second and third trimester respectively). Classification of atherogenic index values: High risk = 0.24 and above; Medium risk = 0.10 – 0.24; Low risk = -0.3 – 0.99 for cardiovascular diseases (Dobiasova, 2006).

4.5 Relationship between Iron Deficiency Anaemiaand Lipid Profile Indices among Pregnant Women Attending Antenatal Care Unit, AKTH, Kano

Pearson/Spearman correlation was conducted between iron indices and lipid profile of pregnant women attending antenatal care unit in AKTH during the second and third trimesters. The results showed significant ($p<0.05$) positive correlationbetween serum iron and cholesterol (0.250), transferrin saturation with cholesterol (0.281) and transferrin saturation with low density lipoprotein (0.215)for pregnant women in their second trimester (Table 4.8). However, there was significant ($p<0.05$) but negative correlation between low density lipoprotein with total iron binding capacity (-0.234) and serum ferritin (-0.221) during the second trimester. Conversely, triacylglycerol, very low density lipoprotein and atherogenic index showed insignificant ($p>0.05$) positivecorrelation with haemoglobin, serum iron, total iron binding capacity, transferrin saturation and serum ferritin, whereas there exist a negative correlation which is not statistically significant ($p>0.05$) between haemoglobin and high density lipoprotein (Table 4.8).

In the third trimester, serum iron showed significant ($p<0.05$) positivecorrelation with triacylglycerol (0.262), very low density lipoprotein (0.264) and atherogenic index (0.316); conversely it showed significant ($p<0.05$) negative correlation with high density lipoprotein (-0.260)as indicated in Table 4.8. There exist a significant ($p<0.05$) positive correlation between transferrin saturation and cholesterol (0.220), triacylglycerol (0.253), very low density lipoprotein (0.254) and atherogenic index (0.348), however there was significant ($p<0.05$) negative correlation between transferrin saturation and high density lipoprotein (-0.313) as shown in Table 4.8. Haemoglobin and total iron binding capacity showed positive and negative correlation respectively with cholesterol, triacylglycerol, low density lipoprotein, very low density lipoprotein and atherogenic index which were not statistically significant ($p>0.05$) as indicated in Table 4.8.

Table 4.8: Relationship between Iron Deficiency Anaemia and Lipid Profile Indices among Pregnant Women across Trimesters Attending Antenatal Care Unit, AKTH, Kano

Trimester	Indices	CHOL	TG	HDL	LDL	VLDL	AI
Second	Hb	0.148	0.136	-0.082	0.128	0.136	0.148
	SI	0.250*	0.180	0.089	0.108	0.178	0.109
	TIBC	-0.223*	0.176	0.017	-0.243*	0.177	0.110
	TS	0.281*	0.080	0.045	0.215*	0.079	0.069
	SF	-0.187	0.122	0.058	-0.221*	0.121	0.067
Third	Hb	0.130	0.165	-0.135	0.118	0.166	0.166
	SI	0.165	0.262*	-0.260*	0.161	0.264*	0.316*
	TIBC	-0.166	-0.048	0.098	-0.158	-0.046	-0.102
	TS	0.220*	0.253*	-0.313*	0.213	0.254*	0.348*
	SF	-0.089	0.131	-0.016	-0.099	0.132	0.090

Values are correlation coefficient (r) of two continuous variables. Values with asterisks (*) are statistically significant at $p<0.05$ (Pearson/Spearman Correlation).

Hb = Haemoglobin;**SI** = Serum Iron;**TIBC** = Total Iron Binding Capacity; **TS** = Transferrin saturation; **SF** = Serum ferritin; **CHOL** = Cholesterol; **TG** = Triacylglycerol; **HDL** = High density lipoprotein; **LDL** = Low density lipoprotein; **VLDL** = Very low density lipoprotein; **AI** = Atherogenic index

4.6 Relationship between Iron Indices among Pregnant Women across Trimesters Attending Antenatal Care Unit, AKTH, Kano

Pearson correlation was conducted between iron indices among the pregnant women attending antenatal care unit in AKTH across the trimesters. The results showed that there was significant ($p<0.01$) positive strong correlation between haemoglobin and serum iron (0.552), haemoglobin with transferrin saturation (0.558) and serum iron with transferrin saturation (0.858), whereas a significant ($p<0.01$) negative correlation was observed between total iron binding capacity and haemoglobin (-0.289) and transferrin saturation with total iron binding capacity (-0.615) in the second trimester as presented in Table 4.9.

Similarly, in the third trimester, transferrin saturation was positively and significantly ($p<0.01$) correlated with haemoglobin (0.476), serum iron (0.878) and serum ferritin (0.365), while transferrin saturation showed significant ($p<0.01$) negative correlation with total iron binding capacity (-0.485) as indicated in Table 4.9.

Table 4.9: Relationship between Iron Indicesamong Pregnant Women across Trimesters Attending Antenatal Care Unit, AKTH, Kano

Trimesters	Indices	Hb	SI	TIBC	TS	SF
Second	Hb	1	0.552**	-0.289**	0.558**	0.182
	SI	0.552**	1	-0.168	0.858**	0.356**
	TIBC	-0.289**	-0.168	1	-0.615**	-0.106
	TS	0.558**	0.858**	-0.615**	1	0.290**
	SF	0.182	0.356**	-0.106	0.290	1
Third	Hb	1	0.512**	-0.070	0.476**	0.122
	SI	0.512**	1	-0.072	0.878**	0.468**
	TIBC	-0.070	-0.072	1	-0.485**	0.005
	TS	0.476**	0.878**	-0.485**	1	0.365**
	SF	0.122	-0.468**	0.005	0.365**	1

Values are correlation coefficient (r) of two continuous variables. Values with asterisks (**) are statistically significant at $p<0.01$ (Pearson/Spearman Correlation).

Hb = Haemoglobin; **SI** = Serum Iron; **TIBC** = Total Iron Binding Capacity; **TS** = Transferrin saturation; **SF** = Serum ferritin

4.7Associationbetween Iron Indices, Lipid Profile and Consumption Pattern of Iron- and Lipid-rich Foodsamong Pregnant Women Attending Antenatal Care Unit, AKTH, Kano

The associationbetween iron indices, lipid profile and consumption pattern of iron-rich and lipid-rich foods among pregnant women attending antenatal care unit, AKTH were established using *Eta* test as presented in Table 4.10 and 4.11. *Eta* test shows association/effect size of a nominal and interval variable on each other with the following definitions:*Eta* value of 0.26 and above indicates large association/effect; value of 0.13 – 0.25 signifies medium association/effect and value of 0.02 – 0.12denotes small association/effect. There were small association/effect(*Eta* test = 0.02 – 0.12)between some foods and different iron and lipid-profile indices while some showed no association. From the data, there was small association (*Eta* test = 0.02 – 0.12) between red meat and haemoglobin, serum iron, transferrin saturation and serum ferritin levels based on weekly consumption as presented in Table 4.10; likewise chicken and fish showed small association (*Eta* test = 0.02 – 0.12) with haemoglobin level according to daily, weekly and monthly consumption pattern; while liver showed no statistical association with serum iron, total iron binding capacity and transferrin saturation levels based on monthly consumption (Table 4.10). Egg showed small association (*Eta* test = 0.02 – 0.12) with total iron binding capacity level whereas beans did not show statistical association with total iron binding capacity based on daily, weekly and monthly consumption. Soybean, pumpkin and spinach showed small statistical association (*Eta* test = 0.02 – 0.12) with serum iron, transferrin saturation and serum ferritin levels using weekly consumption pattern, however moringa has no statistical association with serum iron level based on weekly and monthly consumption (Table 4.10).

Table 4.10: Association between Iron Indices and Consumption Pattern of Iron-rich Foods among Pregnant Women Attending Antenatal Care Unit, AKTH, Kano

Food	Consumption pattern	Iron Indices				
		Hb	**SI**	**TIBC**	**TS**	**SF**
Red meat	DC	0.01	0.00	0.01	0.01	0.00
	WC	0.02*	0.02*	0.01	0.03*	0.03*
	MC	0.01	0.02*	0.00	0.01	0.04*
Liver	DC	0.01	0.01	0.01	0.00	0.01
	WC	0.01	0.00	0.02*	0.01	0.01
	MC	0.02*	0.00	0.00	0.00	0.01
Chicken	DC	0.02*	0.00	0.01	0.00	0.00
	WC	0.05*	0.01	0.03*	0.00	0.00
	MC	0.04*	0.02*	0.04*	0.03*	0.02*
Fish	DC	0.05*	0.01	0.09*	0.03*	0.00
	WC	0.05*	0.01	0.05*	0.04*	0.00
	MC	0.06*	0.02*	0.03*	0.03*	0.01
Egg	DC	0.02*	0.01	0.06*	0.01	0.03*
	WC	0.00	0.00	0.04*	0.01	0.01
	MC	0.00	0.00	0.02*	0.02*	0.01
Beans	DC	0.04*	0.02*	0.00	0.02*	0.02*
	WC	0.03*	0.02*	0.00	0.01	0.09*
	MC	0.00	0.00	0.00	0.01	0.04*
Soybeans	DC	0.01	0.00	0.01	0.01	0.00
	WC	0.01	0.02*	0.01	0.02*	0.02*
	MC	0.01	0.00	0.05*	0.02*	0.04*
Pumpkin	DC	0.00	0.00	0.00	0.00	0.00
	WC	0.01	0.02*	0.03*	0.02*	0.00
	MC	0.00	0.01	0.03*	0.00	0.02*
Spinach	DC	0.01	0.01	0.01	0.01	0.02*
	WC	0.02*	0.02*	0.01	0.02*	0.04*
	MC	0.02*	0.03*	0.00	0.01	0.02*
Moringa	DC	0.00	0.01	0.01	0.01	0.01
	WC	0.01	0.00	0.03*	0.01	0.01
	MC	0.03*	0.00	0.01	0.01	0.03*

Values are *Eta* squared showing association/effect size of a nominal and interval variable on each other. Values with asterisks (*) have small association/effect with the corresponding vertical and horizontal variable (*Eta* Test: 0.26 and above = Large association/effect; 0.13 – 0.25 = Medium association/effect; 0.02 – 0.12 = Small association/effect).

Hb = Haemoglobin; **SI** = Serum Iron; **TIBC** = Total Iron Binding Capacity; **TS** = Transferrin saturation; **SF** = Serum ferritin; **DC** = Daily consumption; **WC** = Weekly consumption; **MC** = Monthly consumption

Additionally in Table 4.11, avocados and nuts showed small statistical association (Eta test = 0.02 – 0.12) with triacylglycerol, low density lipoprotein and very low density lipoprotein levels based on daily consumption. Similarly, coconuts showed small association (*Eta* test = 0.02 – 0.12) with levels of cholesterol, triacylglycerol, low density lipoprotein and very low density lipoprotein according to the monthly consumption pattern (Table 4.11). Olive, vegetable and palm oil were statistically associated with the levels of cholesterol and low density lipoprotein. Moreover, sardine was associated with the levels of cholesterol, triacylglycerol, low density lipoprotein, very low density lipoprotein and atherogenic index; while milk with triacylglycerol, high density lipoprotein levels and atherogenic index were statistically associated based on monthly consumption; yoghurt as well has small association (*Eta* test = 0.02 – 0.12) with cholesterol, triacylglycerol, high density lipoprotein and very low density lipoprotein levels based on weekly consumption (Table 4.11). There was no association between butter and triacylglycerol, very low density lipoprotein levels and atherogenic index, although beef tallow and mayonnaise did show small association (*Eta* test = 0.02 – 0.12) through monthly consumption pattern (Table 4.11).

Table 4.11: Association between Lipid Profile and Consumption Pattern of Lipid-rich Foods among Pregnant Women Attending Antenatal Care Unit, AKTH, Kano

Food	Consumption pattern	Lipid Profile					
		CHOL	**TG**	**HDL**	**LDL**	**VLDL**	**AI**
Avocados	DC	0.01	0.03*	0.00	0.00	0.03*	0.01
	WC	0.01	0.04*	0.01	0.01	0.04*	0.02*
	MC	0.04*	0.01	0.00	0.03*	0.01	0.00
Nuts	DC	0.06*	0.02*	0.00	0.04*	0.02*	0.01
	WC	0.02*	0.01	0.00	0.01	0.01	0.01
	MC	0.01	0.00	0.01	0.01	0.00	0.01
Olive Oil	DC	0.00	0.00	0.07*	0.01	0.00	0.03*
	WC	0.02*	0.02*	0.08*	0.03*	0.02*	0.06*
	MC	0.01	0.02*	0.00	0.02*	0.02*	0.00
Vegetable Oil	DC	0.04*	0.01	0.01	0.02*	0.01	0.01
	WC	0.07*	0.01	0.02*	0.07*	0.01	0.00
	MC	0.01	0.01	0.00	0.02*	0.01	0.01
Palm Oil	DC	0.01	0.02*	0.01	0.02*	0.02*	0.02*
	WC	0.09*	0.01	0.00	0.07*	0.01	0.00
	MC	0.01	0.01	0.02*	0.00	0.01	0.01
Coconuts	DC	0.01	0.01	0.02*	0.02*	0.01	0.01
	WC	0.02*	0.01	0.00	0.01	0.01	0.01
	MC	0.02*	0.02*	0.02*	0.03*	0.02*	0.03*
Milk	DC	0.00	0.02*	0.00	0.00	0.02*	0.00
	WC	0.03*	0.01	0.01	0.02*	0.01	0.01
	MC	0.01	0.02*	0.03*	0.01	0.02*	0.04*
Yoghurt	DC	0.00	0.04*	0.01	0.00	0.04*	0.04*
	WC	0.02*	0.02*	0.01	0.01	0.02*	0.01
	MC	0.02*	0.01	0.03*	0.02*	0.01	0.02*
Butter	DC	0.03*	0.08*	0.05*	0.04*	0.07*	0.08*
	WC	0.00	0.01	0.00	0.01	0.01	0.00
	MC	0.01	0.01	0.01	0.01	0.01	0.01
Sardine	DC	0.00	0.01	0.02*	0.00	0.01	0.01
	WC	0.03*	0.00	0.01	0.04*	0.00	0.00
	MC	0.05*	0.02*	0.00	0.05*	0.02*	0.02*
Beef Tallow	DC	0.00	0.01	0.01	0.00	0.01	0.01
	WC	0.00	0.00	0.02*	0.01	0.00	0.00
	MC	0.00	0.04*	0.00	0.00	0.04*	0.02*
Mayonnaise	DC	0.01	0.04*	0.02*	0.02*	0.04*	0.04*
	WC	0.01	0.01	0.00	0.00	0.01	0.02*
	MC	0.01	0.02*	0.00	0.01	0.02*	0.02*

Values are *Eta* squared showing association/effect size of a nominal and interval variable on each other. Values with asterisks (*) have small association/effect with the corresponding vertical and horizontal variable (*Eta* Test: 0.26 and above = Large association/effect; 0.13 – 0.25 = Medium association/effect;0.02 – 0.12 = Small association/effect). **CHOL** = Cholesterol; **TG** = Triacylglycerol; **HDL** = High density lipoprotein; **LDL** = Low density lipoprotein; **VLDL** = Very low density lipoprotein; **AI** = Atherogenic index; **DC** = Daily consumption; **WC** = Weekly consumption; **MC** = Monthly consumption

CHAPTER FIVE

5.0 DISCUSSION

The socio-demographic factors assessed such as age, level of education and monthly income have been reported to have significant effect on condition and outcomes of pregnancy. Level of education, household monthly income and cultural practices/differences among others might affectresponse and complianceto health and nutrition advice from health-professionals such as iron/folic acid supplementation during pregnancy. These factors may also affect health seeking behavior and eating habits of pregnant women which subsequently have impact on pregnancy and its outcomes. Majority are Hausa because the study area was a Hausa dominant State; AKTH is a tertiary hospital and it is observed that elites are the majority visiting the hospital which could be the reason why majority had tertiary education. The data reported in this study were similar to a study conducted by Nwizu *et al.* (2011) among pregnant women in the same study area where they stated that majority of the pregnant women were within 20-29 years (66.4%), have tertiary education (41.0%)and low socio-economic status (82.7%).

The medical/obstetrics history of the pregnant women were also assessed which revealed that most of them were apparently healthy as at the time of the study.World Health Organisation recommends daily oral iron and folic acid supplementation as part of the antenatal care to reduce the risk of maternal anaemiaand iron deficiency (WHO, 2012). In this present study, 56.4% of the pregnant women reported that they took the iron/folate supplements. The reasons why the women are not compliant with the supplementation could be due to the gastrointestinal side effects or lack of access to free iron/folate supplements. Multiple pregnancies, short recovery between pregnanciesand multiparahave been reported to be risk factors associated with iron deficiency anaemia during pregnancy (Breymann, 2013).From our results, majority of the pregnant women were multigravidaand multiparous which might predispose these pregnant women to development of IDA

if preventive measures are not put in place.The practice of birth spacing among the pregnant women in AKTH is encouraging (WHO recommends at least 24 months interval before attempting the next pregnancy) as majority wait beyond 24 months before attempting the next pregnancy which was similar to the earlier report of Nwizu *et al.* (2011) conducted among pregnant womenattending antenatal care in AKTH, Kano.

One of the sustainablestrategies for preventing IDA isto improve consumption of diversified foods and eating habits in order to enhance iron status and overall health. Daily consumption of foods rich in haem-iron (which is readily absorbed by the body) among the pregnant women was low which might be due to low household monthly income or nausea and vomiting when certain foods are consumed. The present study also reported low daily consumption of non-haem iron source among the pregnant women which is further challenged with low bioavailability. The leading cause of anaemia among pregnant women in West Africa is reported to be inadequate intake of diets rich in iron (McClure et al., 2014; Ononge et al., 2014).Similarly, due to the increased requirement for iron during pregnancy which cannot be met by routine diet, consumption of haem and non-haem iron rich foods is important in order to meet the iron needs of the pregnant mother and the growing foetus. Apart from availability of iron to the pregnant woman and the foetus by consuming iron-rich foods, they also contribute to calories, vitamins (such as vitamins B9 and B12) and minerals (such as zinc and selenium) necessary for healthy pregnancy.

An avenue to overcome low bioavailability of iron from non-haem iron-rich foods is to consume these foods together with enhancers such as vitamins A and C andto avoidtheir consumption together with inhibitors such as coffee/tea and calcium.An enhancer of iron absorption converts the insoluble ferric iron(from non-haem iron food) to the more soluble ferrous iron or maintains the iron released from food breakdown in a soluble form beforeits absorption into the intestinal cellwhile an inhibitor of iron absorption binds non-haem iron to form insoluble complexes which inhibitsiron absorption

into the intestinal cell (Beck, 2016).Result from this study reveals that most of the pregnant women did not consume orangeand carrot as enhancers daily.

There are no specific requirements for lipid consumption during pregnancy though it is recommended that mono- and poly- unsaturated fatty acids should be the major fat consumption (FAO, 2010). Lipids are essential for foetal growth and development as well as assist in adaptation for both the mother and foetus (Pusukuru *et al.,* 2016). It was reported in this study that most of the pregnant women consumed vegetable oil, palm oil, nuts, butter and milkat least 1-3 times daily because the foods are readily available, cheap and tasty. Apart from the dietary sources, synthesis of lipids in the body is increased during pregnancy (Vrijkotte *et al.,* 2012) to meet the requirement of the mother and the developing foetus, this increase (in excess) has been associated with adverse pregnancy outcomes and risk for cardiovascular disease (Mukherjee, 2014).

Anaemia is acritical risk factor during pregnancy which is associated with increased occurrence of maternal and foetal morbidity and mortality (Anlaakuu and Anto, 2017). The prevalence of anaemia, iron deficiency and iron deficiency anaemia among pregnanat women in AKTH, Kano were estimated. The prevalence of anaemia reportedin this study is higher than that reported in a cross-sectional study conducted among pregnant women in AKTH, Kano by Nwizu *et al.* (2011) where14.9% and 37.0% were reported as the prevalence of anaemia in second and third trimesters respectively.Similarly, Gwarzo and Ugwa (2013) reported the prevalence of anaemia of 24.5% among the pregnant womenbased on their study at General Hospital, Dawakin Kudu, Kano. The high prevalence reported in this study could be associated with high proportion of multigravida and multipara among the pregnant women studied or possibly most of the pregnant women entered the pregnancy with low iron stores. Other factors that could contribute to anaemia among pregnant women are dietary pattern, geographical location and season(Stephen *et al.,* 2018).

The result from this study was lower than that obtained among pregnant women in Calabar, Cross-river State which reported 12.5% and 23.3% as prevalence of iron deficiency in second and third trimesters respectively (Okafor *et al.,* 2013). The lower prevalence observed may be due to the fact that majority of the pregnant women in the present study are taking iron/folate supplements.The prevalence of iron deficiency anaemia among the pregnant women based on transferrin saturation and serum ferritin levels was 10.7% and 14.0% respectively, in second trimester which increased to 30.6% and 21.2% during third trimester; this prevalence was reported for the first time among pregnant women in Kano State.The result obtained was similar to that reported by Okafor *et al.* (2013) wherethey found 12.5% and 20.0% prevalence of iron deficiency anaemia in second and third trimesters respectively among pregnant women in Calabar based on soluble transferrin receptor. However, Erhabor *et al.*(2013) reported a lower prevalence of 13.5% and 7.69% based on ferritin levels and transferrin saturation respectively among pregnant women in Sokoto State; likewise 12.3% prevalence of iron deficiency anaemia (based on serum ferritin levels) was found among pregnant women in Lagos State (Ajepe *et al.,* 2020). The prevalenceof IDA observed in this study could be linked to mother's low iron stores at the time of conception and the quantity of iron absorbedas the gestation progresses (Osungbade and Oladunjoye, 2012). It might also bethe low consumption of bioavailable ironas observed in their consumption pattern that is responsible for the iron deficiency or infections such as malariaand hookworm infestation (Cao and O'Brien, 2013).

Furthermore, it was observed in this study that there was decrease in serum iron indices investigated as pregnancy progresses except total iron binding capacity which significantly increased from second to third trimester. Total iron binding capacity is the maximum concentration of iron that can be bound by serum proteins (specifically transferrin) and its levels increase during iron deficiency and pregnancy. These changes might be due to the fact thatiron requirements increases remarkably throughout the second half of gestation because of the expansion of the erythrocyte mass and the

transfer of high amounts of iron to the developing foetus and placental structures (Bothwell, 2000). Moreover, the severity of anaemia also worsens as pregnancy progresses.There exists dramatic change in haemoglobin concentrations during pregnancy in response to the increasing maternal blood volume and the iron requirements of the foetus (WHO, 1989). This study recorded higher proportion of severe and moderate anaemic pregnant women in the third trimester than in the second trimester; although it has been recognizedthat the level of haemoglobin declines in the first trimester, attaining its lowest point in the second trimester, and rises again in the third trimester (Geneva, 2011).

Normal alterations do occur in lipid metabolism duringpregnancy which is essential for foetal growth and development. Normal pregnancy has been linked with projected changes in lipid metabolism and elevations in lipid concentration as pregnancy progresses (Hadden and McLaughlin, 2009; Vrijkotte *et al.,* 2012). This study also investigated the changes in lipid profile prevalence of dyslipidaemia and atherogenic index from second to third trimester among the pregnant women attending antenatal care unit in AKTH, Kano. It was observed that there was significant increase in the levels of cholesterol, triacylglycerol, low density lipoprotein, very low density lipoprotein and atherogenic index in third trimester when compared with the levels recorded duringthe second trimester.These alterations are essential and correspond to clinical manifestations that lead to constant elevation in maternal lipid requirements from preconception to third trimester (Grimes and Wild, 2000; Jin *et al.,* 2016). Theelevations in serum lipid concentrationsobserved in this study mayhave been affected by increase in hormones such as oestrogen and progesterone (Desoye*et al.,* 1987; Pusukuru *et al.,* 2016).

Furthermore, the concentration of high density lipoprotein was lower in the third trimester than in the second trimester among the pregnant women investigated in this studywhich was in accordanceto the findings of Brizzi *et al.*(1999) where the levels of high density lipoprotein during pregnancy initially increased and then dropped in the third trimester. In recent times, there is an increasing global

prevalence of dyslipidaemia which is considered to be of public health concern. Prevalence of dyslipidaemia varies extensively based on physiological state of the body, ethnic group, dietary pattern, socio-demography, cultural practices and economic status (Al-Duais and Al-Awthan, 2019). To the best of our knowledge, this study reported the prevalence and pattern of dyslipidaemia among pregnant women in Kano State for the first time. The prevalence found in third trimester (91.8%) from this study was higher than what was obtained in other studies conducted on dyslipidemia among pregnant women (Feitosa *et al.,* 2017;Sharami *et al.,* 2019) which reported about 83% as prevalence of dyslipidaemia. Similarly, the prevalence of hypertriglyceridaemia, low HDL and elevated LDL found in this study was higher than what were reported by Feitosa *et al.* (2017) and Sharami *et al.* (2019); whereas that of hypercholesterolaemia was similar with prevalence of about 54%. The higher prevalence of dyslipidaemia reported in this study could be attributed to the fact that most of the pregnant women consume food containing saturated lipids coupled with lipid accumulation and occurrence of hyperlipidaemia associated with pregnancy due to increasein oestrogen and progesterone (Pusukuru et al., 2016).

Upon establishing the dyslipidaemia, the risk factor for cardiovascular disease was further assessed among the pregnant women studied using atherogenic index which is a marker that predicts the risk of atherosclerosis and coronary heart disease. This study observed high risk for cardiovascular disease among the pregnant women (81.0%) in second trimester which further increased to 85.0% in the third trimester. The observed high risk for cardiovascular disease among the pregnant women in both trimesters could be linked to the high prevalence of dyslipidaemia recorded which was probably caused by the frequent consumption of saturated lipids. Several studies have reported significant correlation between atherogenicindex and other cardiovascular risk factors (Nansseu*et al.,* 2016; Niroumand*et al.,* 2015). It was reported that thestrong association of atherogenic index with lipoprotein particles may be responsible for its high predictive value ofcardiovascular

diseaseoccurrence (Dobiasova, 2006). It is therefore worth mentioning that atherogenic lipid profile in pregnancy has been linked with increased risk of negative pregnancy outcomes such as preterm delivery, maternal morbidity and mortality (Vrijkotte *et al.,* 2012). A strong association has been reported between dyslipidemia and development of hypertensive disorders of pregnancy as well as intrauterine growth restriction and death of foetus (Singh *et al.,*2018).

Moreover, different studies probing on how lipid metabolism is affected in conditions of iron deficiency have generated inconsistent outcomes (Ozdemir *et al.,*2007). Currently, there is no available information or report on the relationship between iron deficiency anaemia and lipid profile among pregnant women. This study reported for the first time the relationship between some iron and lipid profile indices among pregnant women.It was revealed in this study that among the pregnant women, serum iron and transferrin saturation positively correlates with cholesterol, triacylglycerol, low density lipoprotein, very low density lipoprotein and atherogenic index.However, total iron binding capacity and serum ferritin had significant negative correlation with cholesterol and low density lipoprotein.Conversely, imbalances in serum iron indices following derangement in levels of serum lipids have been reported to serve as risk factors for myocardial infarction and coronary artery disease (Magnusson *et al.,* 1994; Salonen *et al.,* 1992). Vari *et al.* (2007) recorded similar observation withthat of our findings which indicated a significant positive correlation between iron stores and lipid profile.Ozdemir *et al.* (2007) showed that triacylglycerol, total cholesterol and low density lipoprotein levels are reduced in premenopausal women with iron deficiency anaemia. Similarly, reduced amounts of total cholesterol, triacylglycerol, high density lipoprotein, low density lipoprotein and very low density lipoprotein were detected in patients with anaemia compared to the normal controls (Sandeep *et al.,* 2014).

Contrariwise, a cross-sectional study among Chinese adults found that high levels of serum ferritinwasassociated with the prevalence of dyslipidemia and a significant positive association

between serum ferritin levels and lipid parameters (Li *et al.,* 2017). Similarly, Zaribaf *et al.* (2014) reported that there was significant positive correlation between serum ferritin concentration and thatof total cholesterol and triacylglycerol among women of reproductive age. Furthermore, Chopra *et al.* (2015) indicated an uninterrupted association between the levels of serum iron and ferritin with risk factors of coronary artery disease and the progression of atherogenic lipid profile (Toescu *et al.,* 2002). It has been proposed that iron plays a crucial role in the pathogenesis of atherosclerosis, mainly by serving as a catalyst for the atherogenic modification of low density lipoprotein (Minqin *et al.,* 2005; Yuan and Li, 2003). Be that as it may, lipid metabolism is principally influenced by iron deficiency because iron serve as a vital cofactor in several key enzymes involved in metabolism of lipids such as lecithin cholesterol acyltransferase (LCAT), lanosterol-14α-demethylase, carnitine acyl transferase I, etc.

Lastly, the association between iron indices, lipid profile and consumption pattern of iron- and lipid-rich foods among the pregnant women studied were determined. From the result, little association/effectof iron- and lipid-rich foods was observed on the levels of iron indices and serum lipid profile determined among the pregnant women. Some of the iron-rich foods (such as red meat, chicken, fish, egg, soybean, pumpkin and spinach) and lipid-rich foods (like nuts, olive oil, coconuts, yoghurt and beef tallow) contributewithin the range of 2 – 9% to the levels of the iron indices and lipid profile estimated respectively while others had no association.The little effect of dietary iron observed may be linked to low bioavailability of non-haem iron source and low consumption of haem-iron source among pregnant women. The high consumption of coffee/tea recorded among the pregnant women may also inhibit/limit the amount of iron absorbed by the body although it was not recorded whether the coffee/tea was consumed alongside the non-haem iron sources or otherwise.Apart from the normal synthesis of lipids occurring in the body, dietary sources are also important to provide essential lipids such as mono- and poly-unsaturated fatty acids required for the

development and effective functioning of vital organs during gestation. However, saturated lipids from dietary sources may pose threat to the health of mother and developing foetus and could increase the risk of dyslipidemia associated with pregnancy.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 Conclusion

In conclusion;

i. The study showed that majority of the pregnant women investigated were within 19-28 years, full-time housewives, iron/folate compliant, multigravida and multipara.

ii. The prevalence of anaemia in second and third trimesters was of moderate and severe public health significance respectively, while the prevalence of iron deficiency anaemia was 14.0% and 21.2% in second and third trimesters respectively.

iii. The study reported significant reduction in the levels of serum iron indices except total iron binding capacity which increased from second to third trimester.

iv. There was significant increase in the lipid profile indicesacross the trimesters, except high density lipoprotein.

v. Majority of the women were dyslipidaemic andshowed high risk for cardiovascular disease in both trimesters.

vi. The study reported that serum iron and transferrin saturation positively correlate with cholesterol, triacylglycerol, low density lipoprotein, very low density lipoprotein and atherogenic index, while total iron binding capacity and serum ferritin had significant negative correlation with cholesterol and low density lipoprotein.

vii. The study also reported that consumption pattern of iron- and lipid-rich foods among the pregnant women investigated had little association/effect on the amount of serum iron indices and lipid profile parameters measured.

6.2 Recommendations

i. It is recommended that pregnant women attending antenatal care in AKTH should be continuously encouraged on the need to take iron/folate supplements and to create awareness on iron-rich foods, foods that enhance iron absorption and good/hygienic feeding practices in order to combat anaemia and iron deficiency.

ii. Thereis need to includelipid profiling as well as diagnosis of iron deficiency and IDA as part of the regular antenatal care in hospitals so that appropriate management likewise preventive and treatment measures will be put in place as early as possible.

iii. Further studies should be conducted in other States of the country (especially the Northern region) to detect the extent of these problems among pregnant women.

iv. The practice of daily oral iron and folate supplementation among pregnant women in the study area has room for improvement and also the health workers should continue to encourage and enlighten the pregnant women on the benefits of the supplementation to both mother and growing foetus.

v. There is need for more awareness among the pregnant women in AKTH on the benefits of enhancers of iron absorption as well as the disadvantages of consuming iron-rich foods together with inhibitors.

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APPENDICES

Appendix 1a: Ethical Approval from Aminu Kano Teaching Hospital, Kano

AMINU KANO TEACHING HOSPITAL
P.M.B. 3452, ZARIA ROAD, KANO.
(☎. 07068297399, 07068297396) www.akth info/www.akth.org.ng, E-mail. equiries@akth into/akthkano@yahoo.com

CHAIRMAN BOARD OF MANAGEMENT
HON. EMMA ENEUKWU

CHIEF MEDICAL DIRECTOR
PROF. AMINU ZAKARI MOH'D.
MBBS. FMCPath

CHAIRMAN M.A.C
DR. ABDURRAHMAN ABBA SHESHE
MBBS. FMCS. FICS

DIRECTOR OF ADMINISTRATION
ZAINAB A. GWADABE (MRS)
B.A ED, M.ED ADMIN(PLANNING)

NHREC/21/08/2008/AKTH/EC/2550

AKTH/MAC/SUB/12A/P-3/VI/2650 **10th July, 2019**

Muhammad Auwal Saliu
Department of Biochemistry
ABU, Zaria.

Ufs:

The Head of Department
Biochemistry
ABU, Zaria.

ETHICS APPROVAL

Further to your application in respect of your research proposal titled "Prevalence of Iron Deficiency Anaemia and its Relationship with Lipid Profile among Pregnant Women Attending Aminu Kano Teaching Hospital, Kano", The Committee reviewed the proposal and noted same as a prospective study.

In view of the above, Ethics approval is hereby granted to conduct the research.

However, the approval is subject to periodic reporting of the progress of the study and its completion to the Research Ethics Committee.

Regards,

Abubakar S. Mahmud
Secretary, Research Ethics Committee
For: Chairman

Appendix 1b: Ethical Approval from Ahmadu Bello University, Zaria

DIRECTORATE OF ACADEMIC PLANNING & MONITORING
AHMADU BELLO UNIVERSITY, ZARIA
Vice Chancellor: Prof. Ibrahim Garba, B.Sc. (Hons) Geology, M.Sc. (Mineral Exploration) A.B.U., Ph.D Geology (London), D.I.C., FNMGS
Director: Prof. A.A. Tijjani, B.Sc. (Hons) UNIMAID, M.Sc. (UI), Ph.D, D.I.C. (London), FNMS
Tel. Zaria + 234-069-551912 Fax. 234 (069) 550032 Telegram UNIBELLO Zaria e-mail: ...

Appl No.: ABUCUHSR/2020/Biochemistry/015
Approval No: ABUCUHSR/2020/015

26th February, 2020

Dr. A. Salihu,
Department of Biochemistry,
Faculty of Life Science,
Ahmadu Bello University,
Zaria

Dear Sir,

PROVISIONAL APPROVAL OF RESEARCH TITLED 'IRON DEFICIENCY ANAEMIA AND SERUM LIPID PROFILE OF PRGNANT WOMEN ATTENDING AMINU KANO TEACHING HOSPITAL (AKTH), KANO, NIGERIA'

This is to convey the provisional approval of the ABUCUHSR to you for the aforestated study domiciled in the Department of Biochemistry. The approval is predicated on the assumption that you shall maintain and cater for the study subjects as indicated in your application.

Monitoring of the Research by spot checks, invitations, interactions with the subjects by any other means the Committee deems fit shall be undertaken at the convenience of the Committee.

This approval can and shall be revoked should a significant breach in the terms and condition of the approval occur. It is hence your responsibility to ensure that the agreed terms are maintained to the end of the Study.

The said approval shall be posted on the ABUCUHSR Page on the University's website.
Note upon completion of the research, ethical clearance certificate will be issued.

Prof. I.H. Nock
Chairman, ABUCUHSR

Cc. Director, DAPM
" Director, IAIICT
" Head, Department of Biochemistry
" Prof. M. Aminu, Department of Microbiology

Appendix 1c: Ethical Approval from Ministry of Health, Kano State

KANO STATE OF NIGERIA
MINISTRY OF HEALTH
2nd & 3rd Floor, Post Office Road,
P.M.B. 3066, Kano.

Commissioner: 08023337417
Permanent Secretary: 09096619985
website: www.kanostateministryofhealth.gov.ng

MOH/Off/797/T.3/1255 23rd May, 2019

*Ref:*_____ *Date:*_____

Muhammad Auwal Saliu,
Department of Biochemistry,
Faculty of Life Sciences,
Ahmadu Bello University,
Zaria.

RE: APPLICATION FOR ETHICAL APPROVAL

Reference to your letter dated 7th May, 2019 on the above request addressed to the Chairman Health Research Ethics Committee of the Ministry requesting for ethical approval to conduct a Research work at Aminu Kano Teaching Hospital, Kano State.

2. The research entitled ***"Prevalence of Iron Deficiency Anaemia and Its Relationship with Lipid Profile among Pregnant Women Attending Aminu Kano Teaching Hospital, Kano, Nigeria"*** is for the Award of Masters Degree in Biochemistry.

3. In view of the foregoing, I wish to convey the Ministry's approval for you to conduct the research at the above mentioned hospital.

4. You are also requested to share your findings with the Ministry of Health, Kano State.

5. Best Regards

Liti Gwarzo, Abdullahi
DPRS
Secretary (HREC)
For: Honourable Commissioner

Appendix 2: Informed Consent Form

INFORMED CONSENT FORM

I am requesting your voluntary participation in my research which is in partial requirement for the award of master's degree in Nutrition from ABU, Zaria. Please read the following information about the research. If you would like to participate, please sign in the appropriate space below.

Student Researcher: Muhammad Auwal Saliu (Reg Number: P17LSBC8028)

Title of Research: Iron Deficiency Anaemia and Serum Lipid Profile among Pregnant Women Attending Aminu Kano Teaching Hospital (AKTH), Kano, Nigeria.

Aim of the Study: The study is aimed at investigating IDA and serum lipid profile among pregnant women attending antenatal care unit, Aminu Kano Teaching Hospital (AKTH), Kano, Nigeria.

Time required for participation: 25 minutes at two different periods (During second and third trimester).

If you participate, you will be asked to: Answer some questions (close to 20); we will collect your blood sample using needle and syringe by a trained phlebotomist for biochemical analyses of lipid profile, serum iron, total iron binding capacity, serum ferritin and haemoglobin concentration.

Risks: None.

Benefits: There is no direct material or monetary benefit to you. It is an academic research for the purpose of the award of M.Sc. Nutrition from ABU Zaria. However, the study will provide data that will assist government policy formulation that may impact positively and improve the routine antenatal care given to pregnant women in the hospitals.

How confidentiality will be maintained: All information will not be attributed to you and shall not be disclosed to anyone except by your written permission.

If you have any questions about this study, feel free to contact; **My Major Research Supervisor:** Dr. Aliyu Salihu; Department of Biochemistry, ABU Zaria; Telephone: 08030729683.

Voluntary Participation: Participation in this study is completely voluntary. If you decide not to participate there will not be any negative consequences.

Your Consent: By signing this form I am attesting that I have read and understood the information above and I freely give my consent/assent to participate.

Name of Volunteer: _____

Signature & Date: _____

Appendix 1: Validated Semi-Structured Questionnaire Form

Questionnaire on socio-demographic data, consumption of iron-rich and lipid-rich foods and medical/obstetrics history of pregnant women attending Aminu Kano Teaching Hospital (AKTH), Kano State, Nigeria.

Dear respondent,

I am Master of Science student (M.Sc. Nutrition) from Ahmadu Bello University, Zaria, carrying out a research titled **"Iron Deficiency Anaemia and Serum Lipid Profile of Pregnant Women Attending Aminu Kano Teaching Hospital (AKTH), Kano, Nigeria".** Please do answer the questions below correctly. Your response will be treated with confidence and it is purely for research purpose.

Instructions

- In the course of filling this questionnaire, you may be asked to write in number, please enter number as figure rather than words.
- Also, you may be required to write the answer in your own words.
- For questions with options, tick (✓) or circle the option that best applies to you.(Please pick only one from the options)
- Please be honest with all your answers.

Thank you for your understanding.

Section A: Socio-demographic characteristics of the respondent.

Section B: Consumption of iron-rich and lipid-rich foods

Section C: Medical/Obstetrics data.

Hospital/Unit Number: …….

Name of Respondent (Pregnant woman): ……

Address of Respondent: ……

Phone Number: ……..

Date of Assessment: ……

Age: ……

Section A: Socio-Demographic Data.		
Q1.	Total number of people in your household?	…….
Q2.	What is your highest level of education?	No formal/Quranic education =1 Primary =2 Junior secondary =3 Senior secondary =4 Tertiary =5
Q3.	Marital Status?	Married =1 Divorced/ Separated =2 Widowed =3 Single = 4
Q4a.	How many times have you given birth (Parity)?	……

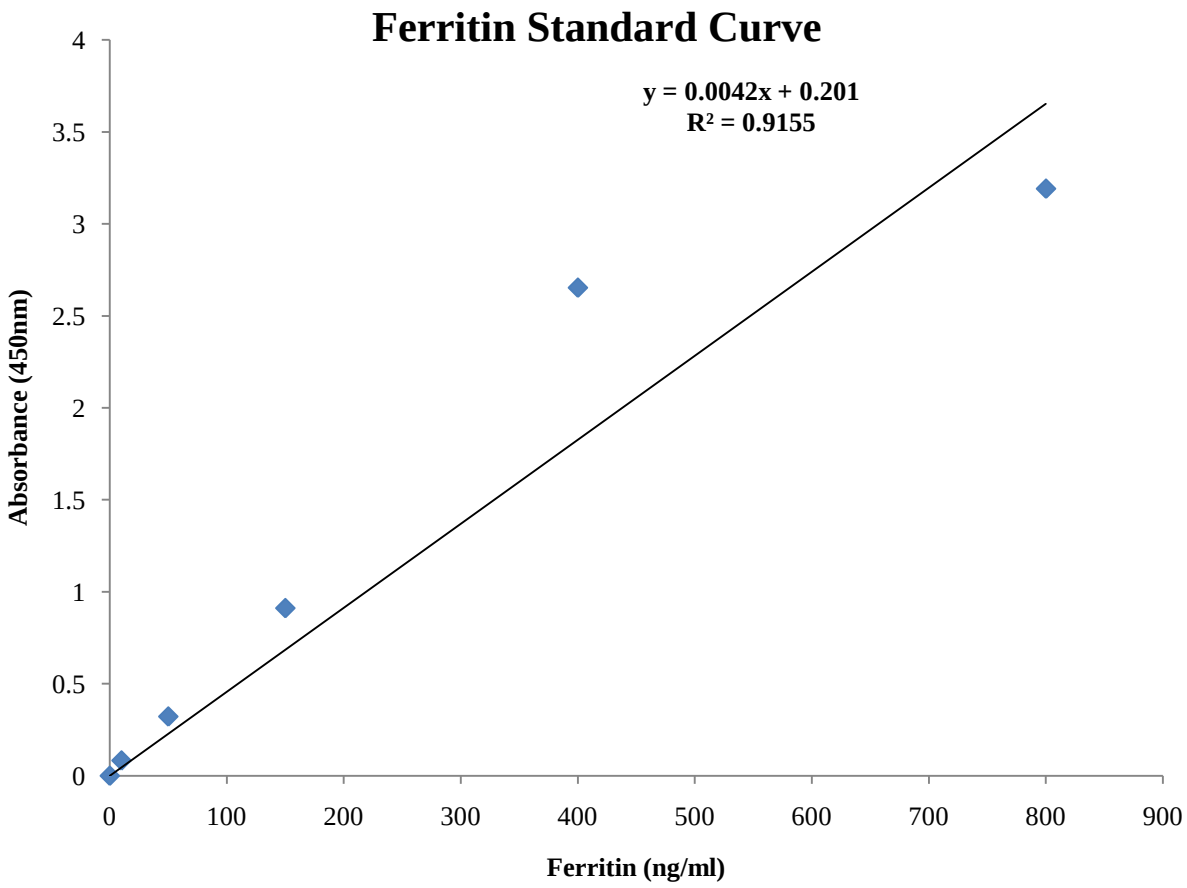
Q4b.	How many children do you have?	……
Q5.	What is your occupation?	Full house wife = 1 Artisan/Skilled workers = 2 Civil servant = 3 Trader = 4 Farmer = 5 Others (Specify) = 6 ……
Q6.	Average household monthly income?	< ₦10,000 = 1 ₦10,000 – ₦19,999 = 2 ₦20,000 – ₦29,999 = 3 ₦30,000 – ₦39,999 = 4 ₦40,000 – ₦49,999 = 5 ≥ ₦50,000 = 6
Q7.	Ethnic nationality?	Hausa = 1 Igbo = 2 Yoruba = 3 Others (Specify) = 4 …….

Section B: Consumption of Iron-rich and Lipid-rich foods										
Q8.	Iron-rich foods	Daily			Weekly			Monthly		
	(Animal source/Haem iron)	Never	1-3	4 or more	Never	1-3	4 or more	Never	1-3	4 or more
	Red meat	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	Liver and other organ meat	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	Chicken (Poultry)	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	Egg	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	Fish	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	(Plant source/Non-haem iron)									
	Beans	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	Soybeans	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	Pumpkin	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	Spinach	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	Moringa	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	Enhancers									
	Oranges	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	Lettuces	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	Carrots	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	Sweet potatoes	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	Inhibitors									
	Coffee/Tea	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Q9.	Lipid-rich foods									
	Avocados	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	Nuts	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	Olive oil	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	Vegetable oil	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	Palm oil	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	Coconuts	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	Milk	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	Yoghurt	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	Butter	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
	Sardines	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]

	Beef tallow Mayonase	[ ] [ ] [ ] [ ] [ ] [ ]	[ ] [ ] [ ] [ ] [ ] [ ]	[ ] [ ] [ ] [ ] [ ] [ ]

Section C: Medical/Obstetrics Data		
Q10.	Have you been diagnosed with any of the following diseases? Chronic kidney disease, Cardiovascular diseases, Cancer, Diabetes, Hypertension, HIV/AIDS, Sickle cell anaemia, Haemoglobinopathies, Hepatitis. Others ……..	Yes = 1 If Yes, please circle the disease No = 2
Q11.	Have you been diagnosed with any disease apart from those listed above?	Yes = 1 If Yes, (Specify)…….. No= 2
Q12.	Are you currently taking any drug/medication?	Yes = 1 If Yes, Go to Q13. No = 2 If No, Go to Q15
Q13.	If yes to Q12, Circle the drug/medication(s) Hydroxyurea, Anti-retroviral drug, Anti-hypertensive drug, Anti-cancer drug, Anti-diabetic drug, Anti-hepatitic-viral drug, Cardiac medications, Iron/folic acid supplement, Vit C supplement, Calcium supplement, Multivitamin supplement.	Others ……. ……. …….
Q14.	Who prescribed the drug/medication you are taken currently?	Doctor = 1 Nurse = 2 Pharmacist = 3 Chemist/Shop = 3 House member = 4 Neighbour = 5 Others (Specify) = 6 ……
Q15.	How old (gestational age) is your pregnancy?	……. weeks
Q16.	Have you had abortion/miscarriage before?	Yes = 1 No = 2
Q17.	What is the duration of your child spacing?	Not applicable = 0 < 12 months = 1 12 – 24 months = 2 > 24 months = 3

Appendix 4: Ferritin Standard Curve



Ferritin standard curve used for the calculation of unknown ferritin concentration in the samples

Appendix 5: Contributions to Knowledge

The study established that:

i. 56.4%, 83.0% and 67.8% of the pregnant women in the study area were iron/folate supplements compliant, multigravida and multipara respectively; while 41.1% had experienced miscarriage/abortion.

ii. prevalences of anaemia, iron deficiency and IDA based on transferrin saturation and serum ferritin levels among the pregnant women during second trimester were 37.5%, 8.0%, 10.7% and 14.0% respectively which significantly ($p<0.05$) increased to 65.8%, 12.9%, 30.6% and 21.2% respectively in the third trimester.

iii. the proportion of mildly anaemic pregnant women dropped to 12.9% in the third trimester while the moderately and severely anaemic ones increased to 40% and 12.9% respectively; only 34.2% were normal based on WHO classification (Normal ≥ 11.0 g/dl; mild anaemia = 10.0 – 10.9 g/dl; moderate anaemia = 7.0 – 9.9 g/dland severe anaemia = < 7.0 g/dl).

iv. the prevalence of dyslipidaemia among the pregnant women studied was 69.6% and 91.8% in second and third trimesters respectively whereas the risk for cardiovascular disease among the pregnant women was 81.0% and 85.0% in second and third trimesters respectively.

v. the prevalence of hypercholesterolaemia, hypertriglyceridaemia, high LDL and low HDL among the pregnant women during the second trimester were 19.6%, 36.6%, 18.8% and 49.1% respectively, which subsequently increased to 54.1%, 75.3%, 40.0% and 62.4% respectively during the third trimester.

vi. serum iron and transferrin saturation positively correlate with CHOL, TG, LDL, VLDL and AI, while total iron binding capacity and serum ferritin had significant negative correlation with CHOL and LDL.