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RESEARCH ARTICLE

FERRITIN CONCENTRATION FOR THE ASSESSMENT OF IRON STATUS AND INFLUENCE OF SOCIO-ECONOMIC STATUS IN PREGNANCY IN SOKOTO

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ABSTRACT

Background; Anaemia is a major health problem in developing countries like Nigeria. It has been implicated as one of the causes of maternal morbidity and mortality. Serum ferritin is generally considered the best test to assess iron status in pregnancy, although it is an acute phase reactant. ***Aim of the study:*** The aim of this study was to determine the serum ferritin and its relationship with socio-economic status in pregnancy. ***Materials and Methods*****:** Serum Ferritin concentration was estimated by a sandwich Enzyme-Linked immunosorbent Assay.The information's from the questionnaires were used to group the pregnant women into their different socioeconomic status. Occupation, Educational level and income were the commonly used indicators of socio-economic classes. ***Results*****:** Low (95.7%) and middle (78.0%) socio-economic class were observed to have anaemia (serum ferritin < 15µg/L) compared to high socio-economic class (35.9%). The difference was statistically significant ($p < 0.001$). ***Conclusions****:* The index study shows that, anaemia was significantly associated with socio-economic status ($p< 0.001$). Also, it could be recommended that, serum ferritin should be included in the routine antenatal investigation for the assessment of iron status, as it reflects the true iron store. This will help in making early diagnosis to prevent the complications of iron deficiencyanaemia.

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INTRODUCTION

Pregnancy is a physiological condition and usually has no effect on general health of a pregnant woman (Chapman *et al.,* 1998). However, it can result to hormonal, haemodynamic and haematological changes. These changes should be viewed as normal, determined by nature (Bolarin, 2013). Increased total blood volume and haemostatic changes can help reduce the hazards of haemorrhage during delivery (Hytten, 1986). Iron deficiency is one of the most prevalent nutrient disorder that affect pregnant women (Raza *et al.,* 2011). It affects approximately two billion people worldwide and is the commonest cause of anaemia (Stevens *et al.,* 2013). Pregnant women in southern part of Nigeria have depleted iron stores and are at risk of developing iron deficiency anaemia (Idowu *et al.,* 2007). The lowest normal haemoglobin in the healthy non-pregnant woman is defined as 12 g/dl (W.H.O., 2011). The World Health Organization recommends that haemoglobin should be maintained at or above 11.0 g/dl, and should not be allowed to fall below 10.5 g/dl in the second trimester (Khusun *et al.,* 1999). Pregnancy usually induces a slight increase (2–3 fL) in mean red corpuscular volume (MCV), independent of folate status. This is sometimes enough to mask the microcytosis normally found in iron deficiency anaemia (Khusun *et al.,* 1999). The iron requirement during the first ten Weeks of gestation is less than that for non-pregnant women, but increases several folds during the last ten Weeks (Svanberg 1975). Because iron deficiency may affect pregnancy outcome, iron status should be assessed during pregnancy (Skikne and Baynes, 1994). Available markers however, tend to be less reliable in pregnancy because they are either not sensitive enough or are affected by gestation, independent of iron status (Cook and Skikne, 1989). For instance, despite an increase in red blood cell mass, the haemoglobin concentration often decrease during pregnancy as a result of expansion of plasma volume (Yip, 2000). Furthermore, haemoglobin is not a sensitive index of mild iron deficiency anaemia and therefore, is not specific for anaemia caused by iron deficiency (Hallberg, 1992). Despite this, haemoglobin measurements are fast, simple, and suitable for screening. Serum ferritin is generally considered to be a more sensitive index of iron status because low concentration correlates well with depleted iron stores (van den Broek, 1996).

Statement of the problem: Anaemia is a major health problem in developing countries (Erhabor *et al.,* 2013). It has been implicated as one of the causes of maternal morbidity and mortality. It also has great effect on the health care system, which results in decrease of quality of life for the affected patients (Isah *et al.,* 2011). Early diagnosis using serum level of ferritin and effective treatment of anaemia will prevent the complication of iron deficiency. The role of serum ferritin estimation as a marker for assessing iron status in general population has been established (Cook and Finch, 1979). However, there is less documented study in our environment on the estimation of serum ferritin in pregnant women. Ferritin kits could be relatively expensive, but using it in the diagnosis of iron deficiency anaemia will reduce maternal mortality. Considering the fact that serum level of ferritin increases during inflammations, however, using it as a marker in pregnancy can help to reduce the rate of hospital admission due to iron deficiency anaemia.

Justification: Iron deficiency is the most common deficiency state in the world, although it is particularly prevalent in developing countries. Effective management is needed to prevent adverse maternal and pregnancy outcomes. However, most of the time clinicians are unable to ascertain the type of anaemia a patient has, because the laboratories have no capacity to carry out the appropriate tests such as serum ferritin. Serum ferritin is generally considered the best test to assess iron deficiency in pregnancy, although it is an acute phase reactant and levels will rise when there is active infection or inflammation.

Aim and objectives

Aim: To determine the serum levels of ferritin and its relationship, with socio-economic status of the pregnant women.

Objectives

- To determine the serum level of ferritin in pregnant women.
- To determine the socioeconomic status of the pregnant women.
- To establish, the relationship between serum level of ferritin and socio-economic status in pregnant women.
- To determine the validity of other markers for assessing iron deficiency anaemia in pregnant women.

Research Hypothesis

The hypothesis of the study was that, serum levels of ferritin of high socio-economic class pregnant women were within normal value, when compared with middle and low socio-economic class pregnant women.

Literature Review

Anaemia in pregnancy is an important public health problem in Nigeria and the world as a whole (Erhabor *et al.,* 2013). World Health Organization estimates that more than half of pregnant women in the World, have a haemoglobin level of less than (11.0g/dl), the prevalence may however be as high as 56 or 61% in developing countries (W.H.O., 2001). Women often become anaemic during pregnancy because the demand for iron and other vitamins is increased due to physiological burden of pregnancy. The inability to meet the required level for these substances either as a result of dietary deficiencies or infection gives rise to anaemia (W.H.O., 2001). Thirty to fifty percent of anaemia in children and other groups is caused by iron deficiency. Because 1.6 billion people are anaemic, several hundred million manifests with iron deficiency anaemia. As such, iron deficiency is the most common cause of anaemia worldwide (W.H.O., 2014). Iron deficiency anaemia afflicts a subset of the two billion people worldwide who are nutritionally iron deficient. Therefore, the health burden of iron deficiency may be extrapolated from the global prevalence of anaemia (Brabin *et al.,* 2003). Anaemia is not distributed evenly throughout the world, as there is a fivefold increase in underdeveloped geographies. In some global regions, the prevalence of anaemia among young children is greater than 50% and even approaches 100% in some locales (Gomber *et al.,* 2003). Serum ferritin is considered to be the best measure of body iron stores in healthy individuals currently available, this despite a day-to-day variation of up to 25% (Herbert *et al.,* 1997). According to Leggett et al. (1990) alcohol consumption may increase serum ferritin levels. Ferritin is an acute phase protein; therefore, serum ferritin is not a sensitive indicator of iron stores in those suffering from an infection, inflammation or malignancy (Kell and Pretorius, 2014).

Functions of Iron: Iron is an essential vital element in all aerobic organisms, it plays vital functions in the human body, largely attributable to its oxygen-carrying capacity. The amount of iron present in a healthy adult is two to four grams (Gueri *et al.,* 1982). Iron is a binding site for oxygen in heme-containing proteins, such as haemoglobin in red blood cells and myoglobin in muscle (W.H.O., 2014). Heme consists of a ferrous (Fe^{2+}) iron complex within protoporphyrin ix. Porphyrin is a cyclic macro - molecule that binds divalent and trivalent metals like iron to form complexes. Thus, haemoglobin and myoglobin performed important function of oxygen transport in human body (Skikne and Baynes, 1994).
Iron is an active transition metal that exists mainly in two oxidative states, ferrous (Fe^{2+}) and ferric (Fe^{3+}) (Auffan *et al.,* 2008). Iron actively participates in electron transfer chains by reducing ferric iron to ferrous iron and supplying proton (H^{+}) ions to the cytochromes. Heme, therefore, is an essential component of cytochrome proteins, which comprise the electron transport chain (Burtis and Bruns, 2014)). In addition, iron is also an integral part of iron-sulfur (Fe-S) proteins, which are present in mitochondrial enzymes, thus playing an important role in mitochondrial single electron transport (Sykes *et al.,* 1995).

Iron requirement during pregnancy: Iron deficiency can be defined as that moment when body iron store become depleted and a restricted supply of iron to various tissue becomes apparent (Bothwell, 2000). The process of iron storage depletion can occur rapidly or very slowly and is dependent on the balance between iron intake and iron requirements (Salvail and Massé, 2012). Pregnancy increases the iron requirement to nearly 6mg/day by the second and third trimester due to the high growth rates of the placenta and fetus and the expansion of maternal red blood cell mass (Bothwell, 2000). Iron absorption increases in individuals who have depleted iron stores, it is this internal regulator of percentage absorption that may be more important than any particular constituents of the food supply (Lipschitz *et al.,* 1974).

Iron homoestasis: It is important to maintain normal levels of iron in the body since iron in excess can cause toxicity by contributing to free radical production and oxidative stress (Burtis and Bruns, 2014). The amount of iron loss from the body depends only minimally upon the iron burden. Regulation of body - iron content is therefore achieved almost entirely by modulating the amount of iron absorbed from the upper intestinal (Burtis and Bruns, 2014).

Regulation of iron availability: A number of proteins are involved in the regulation of iron, including transferrin (Tf) and ferritin, which are responsible for iron transport and storage, respectively. These proteins bind iron in the ferric (Fe^{3+}) form, which is non-reactive. This helps to inhibit the reaction of free iron with oxygen, which can lead to free radical formation and cell injury (Andrews, 1999). Iron availability in the body is regulated through intestinal absorption, post-translational regulatory mechanisms, recycling of iron in macrophages and cellular storage of iron (Salvail and Massé, 2012). Iron absorption is regulated by transport of iron through the small intestine which depends on the rate of erythropoiesis and the reduction in body iron stores. Erythropoiesis takes place in bone marrow. The ferrous form of iron is absorbed from the diet and transported by the plasma protein called transferrin, as well as iron recycled by reticuloendothelial macrophages, is taken up by the bone marrow erythroblasts. Excess iron is stored in the form ferritin, primarily in the liver cell (hepatocytes). Erythropoiesis continues to use this stored iron in case of reduced supply or loss of iron (Andrews, 1999).

Iron and its metabolism: Iron metabolism is a chain of chemical reactions that maintained homeostasis at both the cellular and systemic level (Burtis and Bruns, 2014). The control of this reaction is very necessary as iron is potentially toxic metal is also an important part of many aspects of human health and disease (Burtis and Bruns, 2014). Pathologists have been especially interested in systemic iron metabolism because iron is essential for red blood cells, where most of the human body's iron is contained. Understanding iron metabolism is also important for understanding diseases of iron overload, such as hemochromatosis, and iron deficiency, such iron deficiency anaemia. (Bardou Jacquet *et al.,* 2014).

Importance of iron regulation: Iron is an important element for almost all forms of life, ranging from microorganism to mammals. Its importance lies in its ability to mediate electron transfer, In the ferrous state, iron acts as an electron donor, while in ferric state it acts as an acceptor (Kobayashi and Nishizawa, 2012) Thus, iron plays an essential role in the speeding up of enzymatic reactions that involve electron transfer. Proteins can contain iron as part of different cofactors, as iron-sulfur clusters (Fe-S) and heme groups, both of which are assembled in mitochondria (Kautz *et al.,* 2014).

Iron In Cellular Respiration: Animal and human cells require iron which is used to obtained energy in the form adenosine triphosphate (ATP) from multi-step process called cellular respiration, more in particular from oxidative phosphorylation at the cristae of the mitochondrial. Iron is present in the iron-sulfur clusters and heme groups of the electron transport chain proteins that generate a proton gradient that allows (ATP) synthase to synthesize (ATP) (Puig *et al.,* 2005). Heme groups are part of hemoglobin, a protein found in red blood cells that serve to transport oxygen from the lungs to the tissues. Heme groups are also present in myoglobin to store and diffuse oxygen in muscle cells (Hentze *et al.,* 2010).

Transport of Oxygen: Both human and microorganism needs iron for oxygen transport. Oxygen (O2) is required for the functioning and survival of nearly all cell types (mature erythrocytes being one exception) (Conrad and Umbreit, 2000). Oxygen is transported from the lungs to the rest of the body, bound to the heme group of hemoglobin in erythrocytes. In muscles cells, iron binds myoglobin, which regulates its release (Dixon and Stockwell, 2014).

Toxicity of iron: Iron is a potentially toxic metal. Its ability to transfer and accept electrons shown that, it can speed up the conversion of hydrogen peroxide into free radicals. Free radicals can result to the damage of most structures in cells, and leading to cell death (Crichton *et al.,* 2012). When iron is bound to proteins such as heme is safe. Also, there are virtually no truly free iron ions in the cell, since they readily form complexes with organic molecules. However, some of the intracellular iron is bound to low affinity complexes, and is termed labile iron or "free" iron. Iron in such complexes can result to cell damage (Crichton *et al.,* 2012).

Bacterial protection: The body immune system response to systemic bacterial infection by initiating a process known as iron withholding. In case bacteria survive, then theymust obtain iron from their environment (Parrow *et al.,* 2013). Bacteria that causes disease releasing iron binding molecules called siderophoresand latter reabsorbed them to recover iron, from either hemoglobin or transferrin.(Patidar *et al.,* 2013). The more had they work to acquired iron, the better a metabolic price they must pay (Gottschalk, 2012). This means that iron deprived bacteria reproduce more slowly. So our control of iron levels appears to be an important defence against most bacterial infections; there are some exceptions however. Tuberculosis causing bacterium can reside within macrophages which are an iron rich environment and Borrelia burgdorferi utilises manganese in place of iron. People with increased amounts of iron, like hemochromatosis, are more susceptible to some bacterial infection (Johnson and Wessling-Resnick, 2012).

Although this mechanism is an elegant response to short-term bacterial infection, it can cause problems when inflammation goes on for longer. Since the liver produces hepcidin in response to inflammatory cytokines, hepcidin levels can increase as the result of nonbacterial sources of inflammation, like viral infection, cancer, autoimmune diseases or other chronic diseases. When this occurs, the sequestration of iron appears to be the major cause of the syndrome of anemia of chronic disease, in which not enough iron is available to produce enough hemoglobin containing red blood cells (Salvail and Massé, 2012).

Body iron stores: People that are well nourished, and live in developed countries, have about 4 to 5 grams of iron in their bodies. About 2.5g of this, is contained in the hemoglobin needed to carry oxygen through the blood, and the remaining iron complexes with apoferritin and stored in the bone marrow, liver, and spleen (Cassat and Skaar, 2013). The liver's stores of ferritin are the primary physiologic source of reserve iron in the body. The reserves of iron in developed countries tend to be lower in children and women of childbearing age than in men and in the elderly.

Women who must use their stores to compensate for iron lost through menstruation, pregnancy or lactation have lower non - hemoglobin body stores, which may consist of 500mg, or even less (Cassat and Skaar, 2013). About 400mg of iron stores in human body is devoted to cellular proteins that use iron for essentials cellular processes like storing oxygen (myoglobin) or performing energy producing redox reactions (cytochromes)(Ganz and Nemeth, 2011). Iron deficiency initially affects the store iron in the body, and depletion of these stores is thought to be relatively non symptomatic, although some vague and nonspecific symptoms have been reported to be associated with it (Burtis and Bruns, 2014). Since iron is primarily required for hemoglobin, iron deficiency anemia is the primary clinical manifestation of iron deficiency. Iron deficient people will suffer or die from organ damage well before cells run out of the iron needed for intracellular processes like electron transport (Ganz and Nemeth, 2011). Macrophages of the reticuloendothelial system store iron as part of the process of breaking down and processing hemoglobin from engulfed red blood cells. Iron is also stored as a pigment called hemosiderin which is an ill-defined deposit of protein and iron, created by macrophages where excess iron is present, either locally or systemically for example among people with iron overload due to frequent blood cell destruction and transfusions. If the systemic iron overload is corrected, over time the hemosiderin is slowly reabsorbed by macrophages (Parrow *et al.,* 2013).

Mechanisms of iron regulation: Homeostasis of iron human body is regulated at two different stages. Systemic iron levels are balanced by the controlled absorption of dietary iron by the cells that line the interior of the bowels, and the uncontrolled loss of iron from epithelial sloughing, sweat, injuries and blood loss. However, systemic iron is continuously recycled. Cellular iron levels are controlled differently by different cell types due to the expression of particular iron regulatory and transport proteins (Hentze *et al.,* 2010).

Systemic Iron Regulation

Iron uptake in the diet: Absorption of iron in most of the diet is a variable and dynamic process. The amount of iron absorbed compared to the amount ingested is typically low, but may range from 5% to as much as 35% depending on condition and the nature of iron. The efficiency with which iron is absorbed varies depending on the source. Mostly the best absorbed forms of iron come from animal products (Conrad and Umbreit, 2000). Absorption of dietary iron in iron salt form (as in most supplements) varies somewhat according to the body's need for iron, and is usually between 10% and 20% of iron intake. Absorption of iron from animal products, and some plant products, is in the form of heme iron, and is more efficient, allowing absorption of from 15% to 35% of intake (Brune *et al.,* 1989). Heme iron in animals is from blood and heme containing proteins in meat and mitochondria, whereas in plants, heme iron is present in mitochondria in all cells that use oxygen for respiration (McKie *et al.,* 2001). Like other mineral nutrients, most of the iron absorbed from digested food or supplements is absorbed by cells of the duodenum. These cells have specialised molecules that transport iron into the body. Dietary iron can be absorbed as part of a protein, such as heme protein or iron must be in its ferrous (Fe^{2+}) form. An enzyme called ferric reductase is on the brush border of the enterocytes in the duodenum reduces ferric (Fe^{3+}) to ferrous (Fe^{2+}). A protein called divalent metal transporter1 (DMT1), which can transport several divalentmetals across the plasma membrane, then transports iron across the enterocyte's cell membrane into the cell (McKie *et al.,* 2001). These intestinal lining cells can then either store the iron as ferritin, which is accomplished by Fe^{3+} binding to apoferritin or the cell can release it into the body via the only known iron exporter in mammals, ferroportin (Skaar, 2010). Hephaestion, is a ferroxidase that oxidize Fe^{2+} to Fe^{3+} and is mainly found in the small intestine, helps ferroprotein to transfer iron across the basolateral end of the intestine cells. In contrast, ferroportin is post translationally repressed by hepcidin, a 25-amino acid peptide hormone. The body regulates iron levels by regulating each of these steps. For instance, enterocytes synthesize more duodenal cytochrome B (Dactyl), divalent metal transporter(DMT1) and ferroportin in response to iron deficiency anemia. Iron absorption from diet is facilitated in the presence of vitamin C and diminished by excess calcium, zinc, or manganese (Fleming and Sly, 2001). The rate at which iron in human body is absorbed appears to be as a result of variety of interdependent factors, including total iron stores, the extent to which the bone marrow is producing new red blood cells, the concentration of hemoglobin in the blood, and the oxygen content of the blood (Conrad and Umbreit, 2000). The body also absorbs less iron during times of inflammation, in order to deprive bacteria of iron. Recent discoveries demonstrate that hepcidin regulation of ferroportin is responsible for the syndrome of anemia of chronic disease (Ganz and Nemeth, 2011).

Iron recycling and loss: Most of the time, iron in the body is preserved and recycled by the reticuloendothelial system, which breaks down old red blood cells (RBCs). In contrast to iron uptake and recycling, there is no physiologic regulatory mechanism for removing iron from the body. People lose a small but steady amount by gastrointestinal blood loss, sweating and by shedding cells of the skin and the mucosal lining of the gastrointestinal tract. The total amount of loss for healthy people in the developed country, amounts to an estimated average of 1 mg per day for male, and 1.5–2 mg a day for female with regular menstrual periods. People with gastrointestinal parasitic infections, more commonly found in developing countries, often lose more. Those who cannot regulate absorption well enough get disorders of iron overload. In these diseases, the toxicity of iron starts overwhelming the body's ability to bind and store it (Hentze *et al.,* 2010).

Cellular Iron Regulation

Iron import: Most cell types take up iron primarily through receptor mediated endocytosis by the transferrin receptor 1(TFR1), transferrin receptor 2 (TFR2) and GAPDH. Transferrin bound ferric iron is recognized by the transferrin receptor, triggering a conformational change that causes endocytosis. Iron then enters the cytoplasm from the endosome via importer DMT1 after being reduced to its ferrous state (Hentze *et al.,* 2010). Alternatively, iron can enter the cell directly through plasma membrane divalent cation importers such as divalent metal transporter 1 (DMT1). Again, iron enters the cytoplasm in the ferrous state after being reduced in the extracellular space by a reductase such as STEAP2, STEAP3 (in erythrocytes), Dactyl (in enterocytes) and SDR2 (Hentze *et al.,* 2010).

The labile iron pool: Ferrous iron In the cytoplasm, are in a soluble, chelatable state which constitutes the labile iron pool. In this pool, iron is bound to low molecular weight compounds such as peptides, carboxylates and phosphates, although some might be in a free, hydrated form Alternatively, iron ions might be bound to specialized proteins known as metallochaperones. The labile iron pool is potentially toxic due to iron's ability to generate reactive oxygen species. Iron from this pool can be taken up by mitochondria via mitoferrin to synthesize Fe -S clusters and heme groups(Finney and O'halloran, 2003).

The storage iron pool: Iron can be stored in ferritin as ferric iron due to the ferroxidase activity of the ferritin heavy chain. Dysfunctional ferritin may accumulate as hemosiderin, which can be problematic in cases of iron overload. The ferritin storage iron pool is much larger than the labile iron pool(Skikne and Baynes, 1994).

Iron export: Transport of iron from the cells occurs in different cell types, including neurons, erythrocytes, macrophages and enterocytes. The latter two are especially important since systemic iron levels depend upon them. There is only one known iron exporter, ferroportin (Ganti *et al.,* 2002). It transports ferrous iron out of the cell, generally aided by ceruloplasmin and/or Hephaestion (mostly in enterocytes), which oxidize iron to its ferric state so it can bind ferritin in the extracellular medium(Hentze *et al.,* 2010). Hepcidin causes the internalization of ferroportin, decreasing iron export. Besides, hepcidin seems to down regulate both TFR1 and DMT1 through an unknown mechanism (Duce *et al.,* 2010). Another player assisting ferroportin in effecting cellular iron export is the higher order multifunctional, glycolytic enzyme Glyceraldehyde3phosphate dehydrogenase (GAPDH). A specific post translationally modified isoform of GAPDH is recruited to the surface of iron loaded cells where it recruits apo transferrin in close proximity to ferroportin so as to rapidly chelate the iron extruded.

The expression of hepcidin, which only occurs in certain cell types such as hepatocytes, is tightly controlled at the transcriptional level and it represents the link between cellular and systemic iron homeostasis due to hepcidin's role as "gatekeeper" of iron release from enterocytes into the rest of the body (Hentze *et al.,* 2010). Erythroferrone is a hormone that is produce by erythroblast, this hormone inhibits the activities of hepcidin and so increases the availability of iron needed for hemoglobin synthesis (Kautz *et al.,* 2014).

Translational control of cellular iron: Regulation of iron at cellular levels is ultimately controlled at the translational level by iron responsive element binding proteins IRP1 and especially IRP2. However, some control exists at the transcriptional level. When iron levels are low, these proteins are able to bind to iron responsive elements (IREs). IREs are stem loop structures in the untranslated regions (UTRs) of mRNA (Hentze *et al.,* 2010). Both ferritin and ferroportin contain an IRE in their 5' untranslated regions (UTRs), so that under iron deficiency their translation is repressed by IRP2, preventing the unnecessary synthesis of storage protein and the detrimental export of iron. In contrast, TFR1 and some DMT1 variants contain 3' UTR, IREs, which bind IRP2 under iron deficiency, stabilizing the mRNA, which guarantees the synthesis of iron importers (Hentze *et al.,* 2010).

Iron pathology

Iron deficiency

Iron deficiency can result from a variety of causes. These causes can be grouped into several categories:

- Increased demand for iron, which the diet cannot accommodate.
- Increased loss of iron (usually through loss of blood).
- Nutritional deficiency. This can result due to a lack of dietary iron or consumption of foods that inhibit iron absorption, including calcium, phytates and tannins. Black tea steeped for long has high tannins.
- Inability to absorb iron: A common cause of iron deficiency is the widespread use ofacid reducing medications, the strongest of which are proton pump inhibitors (PPIs) such as omeprazole.
- Damage to the bowel lining. Examples of causes of this kind of damage include surgery involving the duodenum, or diseases like Crohn's or celiac sprue which severely reduce the surface area available for absorption.
- Inflammation leading to hepcidin induced restriction on iron release from enterocytes

Iron overload: The body is able to substantially reduce the amount of iron it absorbs across the mucosa. It does not seem to be able to entirely shut down the iron transport process. Also, in situations where excess iron damages the intestinal lining itself (for instance, when children eat a large quantity of iron tablets produced for adult consumption), even more iron can enter the bloodstream and cause a potentially deadly syndrome of iron overload. Large amounts of free iron in the circulation will cause damage to critical cells in the liver, the heart and other metabolically active organs (Fleming and Ponka, 2012). Iron toxicity results when the amount of circulating iron exceeds the amount of transferrin available to bind it, but the body is able to vigorously regulate its iron uptake. Thus, iron toxicity from ingestion is usually the result of extraordinary circumstances like iron tablet over-consumption rather than variations in diet. The type of acute toxicity from iron ingestion causes severe mucosal damage in the gastrointestinal tract, among other problems (Salvail and Massé, 2012).

Chronic iron toxicity is usually the result of more chronic iron overload syndromes associated with genetic diseases, repeated transfusions or other causes. In such cases the iron stores of an adult may reach 50 grams (Liu *et al.,* 2013). Classic examples of genetic iron overload includes hereditary hemochromatosis (HH) and the more severe disease juvenile hemochromatosis (JH) caused by mutations in either the gene RGMc gene, a member of a three gene repulsive guidance molecule family, (also called hemojuvelin (HJV), and HFE2), Hemojuvelin, or the HAMP gene that encodes (an iron regulatory peptide). The exact mechanisms of most of the various forms of adult hemochromatosis, which make up most of the genetic iron overload disorders, remain unknown. So while researchers have been able to identify genetic mutations causing several adult variants of hemochromatosis, they now must turn their attention to the normal function of these mutated genes (Wu *et al.,* 1999).

Proteins in iron metabolism

Ferritin: Ferritin is an iron storage protein characterized by 24 subunits. These subunits comprised of heavy (H) and light (L) chains, which form a hallow apoferritin shell and hold nearly 4500 atoms of iron. Apoferritin binds iron in its ferrous (Fe^{2+}) form and then oxidizes it to ferric (Fe^{3+}) iron. In this form iron is non-reactive and thus, non-toxic to the cells. The H subunit of ferritin has ferroxidase activity, which is an essential characteristic of this iron storage protein. The heavy subunit is found mainly in heart tissue and the light subunit is found in liver cells (Harrison *et al.,* 1991). Once iron is absorbed in the duodenum, it is transported across the enterocyte and is either transported to cells in the transferrin-bond form, or the excess iron is stored in the form of ferritin. When iron is required for erythropoiesis, it is released from ferritin. Thus, ferritin levels can reflect an early stage of iron deficiency in a normal healthy adult and are considered as sensitive biomarker of iron deficiency (Cook *et al.,* 1974). However, chronic inflammatory conditions can alter serum ferritin levels, resulting in a false estimation of iron stores, this can be explained by alteration in ferritin due to the inflammatory response. Ferritin is a positive acute phase protein, which means that its level rise in response to inflammation stimulation of interleukins such as IL-1 and IL-6, which gives rise to increased transcription of ferritin. Thus, chronic inflammatory conditions cause increased serum ferritin level. Inflammatory cytokines can also cause increased serum ferritin levels (Burtis and Bruns, 2014).

Hephaestin: Hephaestin is a ferroxidase enzyme located at the basolateral membrane of the erythrocyte, it facilitates the conversion of ferrous iron (Fe^{2+}) to ferric iron (Fe^{3+}), thus being responsible for iron export from the intestinal cell to the circulation. It is a copper dependent protein (Vulpe *et al.,* 1999).

Hepcidin: Hepcidin is a peptide hormone which is synthesized by the liver cells. It is homologous to anti-microbial defensive peptide present in the human immune system, which explains its potential role in inflammation and defence system (Park *et al.,* 2001). Hepcidin appears to be instrumental in iron metabolism, regulation of hepcidin with ferroportin (Fpn) (Origa *et al.,* 2007). Ferroportin (Fpn) is a protein that transports iron, primarily located at the basolateral membrane of intestinal cells as well as macrophages. Hepcidin binds with (Fpn) at the membrane, and this complex is then internalized and degraded by lysosomal enzymes. As a result, ferroportin (Fpn) degradation inhibits iron export from the intestinal cells. The same mechanism takes place in the macrophages, where the release of iron recovered from senescent red blood cells is inhibited. Hepcidin release from the liver is controlled by the erythropoietic demand and body iron stores. In iron deficiency, Hepcidin is down -regulated, thus enhancing iron efflux by ferroportin (Fpn) into the circulation. When serum iron levels are normal, ferroportin is reduced following increased degradation caused by binding of hepcidin, thus inhibiting excess iron export into plasm (Nemeth, 2008). Hepcidin mechanism in inflammatory conditions has been an important component in understanding the physiology of inflammation and poor iron status. Chronic diseases, bacterial and viral infections and autoimmune diseases trigger immune responses by production of cytokine such as interleukin-1, interleukin-6, interleukin-10, TNF-α, interferon- ɤ (Eijk, 1998). These cytokine causes different responses by iron regulatory proteins, for instance hepcidin is up regulated as a defence system in response to inflammation (Knutson *et al.,* 2005). Elevated hepcidin levels, through the down-regulation of ferroportin, cause iron to be sequestered in enterocytes and macrophages, which inhibits iron release into the plasma. This result in reduce plasma iron levels, reduced erythropoiesis, and consequently anaemia (De Domenico *et al.,* 2007).

Biomarkers for assessing iron status: There are different ways in which iron status can be assessed (W.H.O., 2001). The sensitivity and specificity of these parameters have been studied to identify the most accurate and reliable biomarkers of iron status. If iron depletion is diagnosed at an early stage, preventive measures of iron supplementation can be implemented to improve iron status (Lipschitz *et al.,* 1981).

Bone Marrow Examination: Iron from it storage site, it is taken up by the erythroblasts present in bone marrow. Bone marrow examination includes histological assessment of iron stores. Although it is considered as the most definitive measurement of iron status. However, it is not used for screening of iron deficiency in major population because of its invasiveness. It is mainly used to assess iron status in hospitalized patient (Ganti *et al.,* 2002).

Hemoglobin: Hemoglobin (Hb) is an iron bound protein in circulating erythrocytes (Pollycove and Mortimer, 1961). It is used for screening of anaemia in large populations (Asobayire *et al.,* 2001). However, it is not a reliable measure because it can't differentiate between types of nutritional anaemia. The normal (Hb) level can varies based on different populations as well as age, sex, and hydration status (Milman *et al.,* 2001). The cut off level as per World Health Organization standard is 13g/dl for adult male, 12g/dl for non-pregnant women and 11g/dl for pregnant women (Khusun *et al.,* 1999).

Free Erythrocyte Protoporphyrin (FEP): The heme molecule consists of protoporphyrin ix and iron. In the later stages of iron deficiency, free erythrocyte protoporphyrin level increase as a manifestation of reduced heme synthesis. This is not a sensitive indicator of iron deficiency, since this occurred in late stage of iron depletion, leading to iron deficient erythropoiesis. (Cook and Finch, 1979). The cut -off value for FEP IS 70µg/dl. Increased levels (FEP) indicate iron deficiency anaemia. (Koller *et al.,* 1978).

Mean Cell Volume (MCV): Mean Cell Volume is not sensitive marker to depletion of iron stores, but iron deficiency erythropoiesis and iron deficiency anaemia are associated with microcytosis (small cell volume), so (MCV) measurements are useful in determining the type of anaemia. However, (MCV) can also decrease in infection or chronic disease, leading to a false assessment of iron deficiency anaemia. Therefore, (MCV) alone is not considered a reliable measure of iron deficiency. The normal value for MCV is 80fL (Patidar *et al.,* 2013).

Transferrin Saturation: Transferrin saturation measured how much iron is bound to transferrin and is express in the form of percentage saturation. Transferrin saturation <20% reflects iron deficiency. Similar to other iron related measure, such as Hb, MCV and FEP, transferrin saturation measurements are not sensitive to early stage depletion of iron body stores. therefore, can be a false indicator of iron deficiency (Wish, 2006)

Serum Ferritin: Normal ferritin, concentrations in the serum varies with age and sex. However, concentration is high at birth, rise during the first two months of life, and then fall throughout late infancy (Domellöf *et al.,* 2002). At about one year of age, concentrations begin to rise again and continue to increase into adulthood. (Gibson, 2005). At early adolescence, males tends to have higher values of serum ferritin than the females, a trend that persists into late adulthood. Values among men reaches its peak between 30-39 years of age and then remain steady until about 70 years of age. Among women, serum ferritin values remain relatively low until menopause and then rise (Gibson, 2005). Serum ferritin levels, as against haemoglobin, are not affected by residential elevation above sea level (Milman *et al.,* 2001). However, ferritin is a positive acute phase response protein where by concentrations increases during inflammation and thereby no longer reflect the size of the iron store, this makes the interpretation of normal or high serum ferritin values difficult in areas of widespread infection, inflammation or malignancy (Kalantar-Zadeh *et al.,* 2004). In case there is no inflammation or chronic disease, high serum ferritin concentrations indicate iron overload (Lipschitz *et al.,* 1974).

Serum Transferrin Receptor: Transferrin receptor in serum is largely derived from developing red blood cells. Cells can regulate their iron uptake by modulating the expression of the transferrin receptor on the cell surface and by storing excess iron as ferritin (Skikne, 2008). Therefore, serum transferrin receptor levels reflect the intensity of red blood cell formation, or erythropoiesis, and the demand for iron. As the supply of iron to the tissues progressively decreases, the expression of transferrin receptors increases. The opposite phenomenon is observed in the immune system, where increased cellular iron stores in macrophages raise transferrin receptor expression (Cook *et al.,* 1993). Assessment of serum transferrin receptor levels has been used to distinguish iron deficiency anaemia from anaemia of chronic disease because the receptors are generally unaffected by concurrent infection or inflammation. However, because serum transferrin receptors levels are affected by the rate of erythropoiesis from any cause, they cannot be used as the sole indicator of erythropoiesis due to iron deficiency. For example, serum transferrin receptor levels may be elevated in conditions with increased red cell production and/or turnover, such as thalassaemia and haemolytic anaemia, or decreased in situations of marrow hypoplasia, such as after chemotherapy. Therefore, serum transferrin receptor levels are an indicator of the severity of iron insufficiency only when iron stores are depleted and no other causes of abnormal erythropoiesis are known (Punnonen *et al.,* 1997).

Total iron binding capacity (TIBC): Total iron binding capacity (TIBC) is sometimes called transferrin iron binding capacity, is a marker that assess iron store, it measured the blood's ability to bind iron with transferrin. The maximum amount of iron bind to transferrin is called the (TIBC) and is indirectly measuring the amount of transferrin. The (TIBC) should not be confused with unsaturated iron binding capacity (UIBC) as (UIBC) is calculated by subtracting the serum iron from the total iron binding capacity (TIBC) (CARR, 1971). Total iron binding capacity (TIBC) reference range is between 40-76µmol/L in adult non pregnant woman,42-73µmol/L in first trimester pregnancy,54-93µmol/L in second trimester, and 68-107µmol/L in third trimester gestation.(Burtis and Bruns, 2014). People suffering from iron deficiency anaemia, usually have higher (TIBC) values than normal range. In pregnancy, the value of (TIBC) is elevated as a result of increase production of transferrin by the liver. However, (TIBC) is lower than the normal reference range in patient suffering from hemochromatosis (Pollycove and Mortimer, 1961).

Hematocrit: Hematocrit (HCT) can be also called parked cell volume (PCV) or even erythrocyte volume fraction (EVF), is the volume percentage (%) of red blood cells in blood. The normal value in adult non pregnant women ranges between 38-46%. Is one of the parameters of full blood count together with hemoglobin concentration, white blood cell count, and platelet count. Haemoglobin in the erythrocytes bind and transport oxygen from the lung to the tissue, therefore the percentage volume of the red blood cell can become a point of reference of its capability of delivering oxygen (Balarajan *et al.,* 2012).

Dietary factors affecting iron status

Iron Intake: Heme and non-heme iron are two forms of iron that are found in animal and plant foods. Heme iron is formed from hemoglobin and myoglobin present in animal tissues, such as meat, Seafood and poultry. Non-heme iron can also be found in animal sources, such as egg yolk and milk, as well as in plant sources, including fortified grains and cereals, leafy green vegetables, nuts, oilseeds and legumes (Bolarin, 2013). A study carried out by (Hunt, 2002) assessed non-heme iron absorption from a lactovegetarian diet compared to heme iron absorption. The bioavailability of heme iron (3.8%) was found to be 70% higher than that of nonheme iron (1.1%). This greater bioavailability is likely related to the structure of heme $(Hb)^3$, which facilitates absorption and is unaffected by dietary inhibiting factors that influence absorption of nonheme (Punnonen *et al.,* 1997) These studies indicate that heme iron intake enhances the bioavailability of iron. While this suggests that greater heme iron intake should improve iron status.

Absorption of Nonheme Iron: Non-heme iron absorption is a complex process that involves a number of other nutrients and dietary components that can be broadly classified as enhancers or inhibitors of iron absorption (Yanoff *et al.,* 2007).

Enhancers of non-heme iron absorption

Ascorbic Acid: The absorption of the dietary iron can be enhanced by consumption of ascorbic acid. In previous studies, radioactive iron administered with vitamin C in single meal showed enhanced iron absorption as compared to the long-term supplementation of vitamin C. One such study by (Cook and Reddy, 2001) showed a positive correlation between log of iron absorption and log of vitamin C consumption. Vitamin C is thought to enhance iron bioavailability by reducing insoluble ferric iron into ferrous form, which leads to increased absorption (Cook and Reddy, 2001).

Meat Factor: The bioavailability of non-heme iron can be enhanced by addition of meat to the meal. This was tested in a meal, to which 75g of ground beef meat was added, nearly tripling non-heme absorption from 0.17mg to 0.45mg (Hallberg, 2001). Although the factors that responsible for this enhancing effect have not been identified yet. However, some studies have concluded that isolated protein extracts from muscle proteins present in chicken and beef enhance non-heme

iron absorption by 100% and 180%, respectively (Allen *et al.,* 2006).

Vitamin A: Vitamin A is a fat-soluble vitamin, that is essential for vision, immunity, cellular differentiation and hematopoiesis. In addition, vitamin A supplementation improves iron status in many epidemiologic studies. A study carried out in 1060 Thai children aged 1-8years, which continued later with vitamin A intervention trial, indicated a positive association of retinol with iron status indices. In the intervention trial, vitamin A supplementation improved iron status significantly (Bloem *et al.,* 1989). Another study, conducted in 100 Venezuelan subjects, reported a significant increase in non-heme iron uptake when supplemented with varying concentrations of vitamin A and β-carotene. The meals were composed of rice, wheat and corn. Vitamin A and β-carotene improved the absorption of iron 0.8-, 1.4- and 2-fold for wheat, corn and rice, respectively. The researchers suggested that vitamin A might have a binding effect on iron that makes it soluble and more bioavailable. Others have suggested that vitamin A could be responsible for mobilizing iron from ferritin stores to the circulation, thus enhancing erythropoiesis (García-Casal *et al.,* 1998).

Copper: Copper deficiency and its relation to iron deficiency have been studied to a large extent. In-vivo studies have shown that copper deficiency causes a significant reduction in iron absorption to about 50% (Reeves *et al.,* 2005). The possible underlying mechanism corresponds to altered function of a ferroxidase called Hephaestin situated at the basolateral membrane of the intestinal cell. Hephaestin is responsible for oxidation of $Fe2^{+}$ to $Fe3^{+}$, the latter is then transported into the blood stream. This ferroxidase is copper dependent, which justifies the mechanism of copper deficiency resulting in iron deficiency by restricting the passage of iron from enterocyte to the blood stream, thus leading to reduced serum iron. Deficiency can also adversely affect ceruloplasmin (Cp), which is a copper containing enzyme present in circulation. In other words, it is another form of ferroxidase responsible for iron transport (Reeves *et al.,* 2005).

Inhibitors of iron absorption

Phytates: Grains such as wheat and oats that are not extensively processed have higher bran content. The bran or husk contains high amounts of phytates, which are also known as inositol phosphates. Cereal grains that have high content of phytates affect iron absorption. A study carried out by (Brune *et al.,* 1989) assessed the effect of phytates with different formulations of bread with combinations of different flours, and number of phosphates. The study showed that the higher the number of phosphates in inositol, the greater the inhibitory effect. Inositol phosphate is commonly seen in hexaphosphate or pentaphosphate form. Cooking methods such as fermentation and acidic media can reduce the number of phytates, thus attenuating its hindering effect to some extent. Fermentation breaks down phosphates to inorganic phosphates and reduces the number of phosphates, thus minimizing its effects. Also, the addition of lactic acid might decrease the phytate content in grains (Brune *et al.,* 1989).

Polyphenols: Polyphenols are ring structures that contain more than one hydroxyl group. They are commonly found in tea, coffee, red wine, spinach and spices such as cinnamon. These hydroxyl groups bind to iron and make it unavailable for absorption (Zijp *et al.,* 2000). The inhibitory effect of these polyphenols on iron absorption has been widely studied. One such study performed by (Zijp *et al.,* 2000) examined iron absorption from controlled breakfast meals that included specific doses of tea and coffee. The results showed that a dose-dependent administration of tea and espresso coffee indicated a 50% decrease in iron absorption, this suggests that the consumption of tea and coffee is one of the important determinants of iron absorption (Zijp *et al.,* 2000).

Calcium: Calcium is an element that is very important for bone health (Zhu and Prince, 2012). Although calcium supplementation may be beneficial for maintaining women's bone health, it might also inhibit iron absorption. A research carried out by (Cook *et al.,* 1991) determined the effect of different calcium supplements on absorption of dietary labelled of non-heme iron and supplemental iron in 61 subjects (28 women and 33 men). When supplements containing both calcium carbonate and iron were taken without food, iron absorption was reduced by 15% as compared to 24% when taken with meal. However, when calcium supplement was taken along with food with more bioavailable iron and low in calcium content, iron absorption was reduced up to 28% as against 55% reduced absorption in a meal with less bioavailable iron and high calcium content (Cook *et al.,* 1991).

Sensitivity, specificity and predictive value

Sensitivity: The term sensitivity was introduced by (Yerushalmy, 1947) as a statistical index of diagnostic accuracy. It has been defined as the ability of a test to identify correctly all those who have the disease, that is "true positive". In this context is the tendency of iron marker to detect accurately those with anaemia. A 90 percent sensitivity of the anaemic pregnant women screened by the test will give a "true positive" result and the remaining 10 percent a "false negative" result.

Specificity: It is defined as the ability of a test to identify correctly those who do not have the disease, that is, "true negatives". A 90 percent specificity means that 90 percent of the non-diseased persons will give "true negative" result, 10 percent of non-diseased people screened by the test will be wrongly classified as "diseased" when they are not.

Predictive accuracy: In addition to sensitivity and specificity, the performance of a screening test is measured by its "predictive value" which reflects the diagnostic power of the test. The predictive accuracy depends upon sensitivity, specificity and disease prevalence. The "predictive value of a positive test" indicates the probability that a patient with a positive test result has, in a given population, the more accurate will be the predictive value of a positive screening test. The predictive value of a positive result falls as disease prevalence declines.

Socio-economic status and it classification

Definition of Socio-economic Status: Socioeconomic status (SES) is a tool that is used to measured economic and sociological parameter combined together of a person's work experience and of an individual's family's economic and social position in relation to others, in respect of income, education, and occupation.

When looking at a family SES, the household income, education, and occupation are always taken into consideration (Ogden and Statistics, 2010). Socioeconomic status is classified into three basic classes high, middle and low SES to describe the three areas a family or an individual may likely fall into. When placing a family or individual into one of these categories or all of the three variables (income, education, and occupation) can be assessed. Additional, low income and illiteracy have showed to be good predictors of a range of physical and mental health problems, ranging from respiratory viruses, arthritis, coronary diseases, and schizophrenia. These may be due to environmental conditions in their workplace, or in the case of mental illnesses, may be the entire cause of that person's social predicament to begin with (Winkleby *et al.,* 1992). Education in higher socioeconomic families is typically emphasised as a more important in topics in the household and local community. In a poor community where food and safety are its priority, education is to the last aspect to be considered. While youth audience are particularly at risk for any health and social issues in the United States such as unwanted pregnancies, drug abuse, and obesity (Statistics, 2012).

Main factors used in classifying socioeconomic status.

Income: Refers to wages, salaries, profits, rents and any flow of earnings received. Income can also come in the form of unemployment or workers compensation, social security, pensions, interest or dividends, royalties, trusts, alimony, or other governmental, public, or family financial assistance. Income can be seen in form of relatives and absolute. Absolute income, as theorized by economics John Maynard Kynes, is the relationship in which as income increases so will consumption, but not at the same rate (Zimmerman and Katon, 2005). Relative income dictates a person or family's savings and consumption based on the family's income in relation to others. Income is a commonly used measure of SES because it is relatively easy to assess for most individuals (Berkman *et al.,* 2014). Income inequality is most commonly measured around the world by the Gini coefficient, where zero corresponds to perfect equality and one mean perfect inequality. Low income families focus on meeting immediate needs and do not accumulate wealth that could be passed on to future generations, thus increasing inequality. Families with higher and expendable income can accumulate wealth and focus on meeting immediate needs while being able to consume and enjoy luxuries and weather crises (Berkman *et al.,* 2014).

Education: Also plays a role in income. Median earnings increase with each level of education. The highest degrees, professional and doctoral degrees, make the highest weekly earnings while those without a high school diploma earn less. Higher levels of education are associated with better economics and psychological outcomes (i.e. more income, more control, and greater social support and networking) (Winkleby *et al.,* 1992). Education plays a major role in skill sets for acquiring jobs, as well as specific qualities that stratify people with higher SES from lower socio-economic status SES. An interesting observation that studies have noted is that parents from lower SES households are more likely to give orders to their children in their interactions, while parents with a higher SES are more likely to interact and play with their children.(Berkman *et al.,* 2014). A division in education attainment is thus born out of these two differences in child rearing. Research has shown how children who are born in lower SES households have weaker language skills compared to children raised in higher households (Yang *et al.,* 1996). These language skills affect their abilities to learn and thus exacerbate the problem of education disparity between low and high SES neighbourhoods. Lower income families can have children who do not succeed to the levels of the middle-income children, who can have a greater sense of entitlement, be more argumentative, or be better prepared for adult life (Yang *et al.,* 1996).

Occupation: Occupation is one component of SES, encompasses both income and educational attainment. Occupational status reflects the educational attainment required to obtain the job and income levels that vary with different jobs and within occupations. Additionally, it shows achievement in skill required for the job. Occupational status measures social position by describing job characteristics, decision making ability and control, and psychological demands on the job (Winkleby *et al.,* 1992). Occupations are ranked by the census (among other organizations) and opinion polls from the general population are surveyed. Some of the most prestigious occupations are physicians and surgeons, lawyers and chemical and biomedical engineers, university professors, and communications analysts. These jobs, considered to be grouped in the high SES classification, provide more challenging work and greater control over working conditions but require more ability. The jobs with lower ranking include food preparation workers, counter attendants, bartenders and helpers, dishwashers, janitors, maids and housekeepers, vehicle cleaners, and parking lot attendants. The jobs that are less valued also offer significantly lower wages, and often are more laborious, very hazardous, and provide less autonomy (Hollingshead, 2015).

Occupation is the most difficult factor to measure because so many exist, and there are so many competing scales. Many scales rank occupations based on the level of skill involved, from unskilled to skilled manual labour to professional, or use a combined measure using the education level needed and income involved (Hauser, 1994). In summary, the majority of researchers agree that income, education and occupation together best represent SES, while some others feel that challenges in family structure should also be considered (Hauser, 1994).

Classification of Socioeconomic Status: According to Olusanya et al. (2004) socioeconomic classification is a simple scoring system that derive the social class of the woman by combining the scores assigned to her husband's occupation with that assigned to her level of education. In this classification system, social classes 1 & 2 = "High" social class; social class 3= "Middle" social class; while social classes 4 & 5 = "Low" social class. The various possible combinations and final scores are expressed in the schematic representation below.

MATERIALS AND METHODS

Study Area: The research was carried out in Sokoto State, which is in the north-western part of Nigeria. Sokoto state share boundary with Niger Republic to the North, Kebbi State to the West and South and Zamfara state to the South and East. Sokoto state was founded on the 3rd February, 1976 by former military leader General Murtala Mohammed with an area of

25,973 square kilometres. It has 23 local government areas with an estimated population of 3,696,999 based on 2006 census. Sokoto state is in the dry Sahel surrounded by Sandy Savannah and isolated hills. Annual average temperature can reach 28.3°C (82.9°F), the warmest months are from February to April, where temperature in the day time can be more than 45°C (113.0°F). The months of rainy season are from June to October, while cold season start from late October to February. The study would be basically in the Department of Chemical Pathology and Immunology, College of Health Sciences (CHS) and Department of Obstetrics and Gynaecology Specialist Hospital Sokoto.

Study Design

This is a descriptive cross-sectional study, which was performed on pregnant women attending antenatal clinic at Specialist Hospital Sokoto, for a period of six months. The research was carried out only on pregnant women, and were grouped based on gestational age and socioeconomic status.

Study population: 270 subjects were recruited for the study. Only pregnant women who fulfilled the inclusion criteria and agreed to participate in the study were selected.

Ethical consideration: Approval was obtained from the Ethics and Research Committee of the Specialist Hospital Sokoto.

Informed Consent: Participants (pregnant women) were fully informed, and their consent were obtained before the commencement of the research.

Selection Criteria

Inclusion Criteria

- Pregnant women aged 15 years and above
- Subjects who meet the above criteria and agree to participate in the study by signing the informed consent form.

Exclusion Criteria

Pregnant women with diabetes mellitus.
Pregnant women with acute viral hepatitis.
Pregnant women with haemoglobinopathies
Pregnant women with febrile illness
Pregnant women that consume alcohol.

Selection of subjects and sampling technique

Consecutive sampling method: Pregnant women who satisfied the study inclusion criteria were consecutively selected from the population of pregnant women attending antenatal care at Specialist Hospital Sokoto.

Sample size determination: The number of pregnant women for inclusion in the study was determined using the sample size formular for estimating sample size for descriptive studies (Nkwo *et al.,* 2013).

$n = Z^2 pq/d^2$

where

n= minimum required sample size in population
Z = Standard normal deviation at confident interval (1.96)
P = Prevalence of (0.2) obtained from previous study (Erhabor *et al.,* 2013).
q = Proportion of failure = 1-P = 1-0.2 = (0.8)
d = Precision 5% (0.05)

Given 20% as the prevalence of pregnant women with inadequate iron status and at risk of developing anaemia in pregnancy.

The minimum sample size $n = \frac{(1.96)^2 \text{ X } 0.2 \text{ X } 0.8}{0.05^2}$

$$n = \frac{3.8416 \text{ X } 0.2}{0.01}$$

$$n = \frac{0.614654}{0.0025} = 245$$

The attrition factor of ten percent was included which is

10/100 x245=24

A total number of 270 pregnant women were recruited for the study.

Patient Preparation

Informed consent was obtained from all pregnant women selected for the study.

Sample collection and storage: Blood sample was collected for serum ferritin, serum iron, total iron binding capacity (TIBC), pack cell volume (PCV), Haemoglobin (Hb), and Red cell count estimation. For accurate comparison to established normal values, a fasting morning serum sample was collected. Subjects were seated and rested for about five minutes, (Romanski and Mcmahon, 1999) before blood sample collection. Venous blood was collected from the antecubital veins. five milliliters (ml) of blood was transferred to EDTA tubes for full blood count estimation, the whole blood was stored at the laboratory of Chemical Pathology (UDUTHS), before analysis. Five milliliters (ml) was transferred into plain tubes on ice, for serum ferritin, serum iron, and total iron binding capacity estimation. The blood sample was centrifuged to separate the serum from the cells within 3 hours. Samples were refrigerated at 2-8°C for maximum period of five (5) days. The specimens were stored at temperature of -20°C at the SHS main laboratory, for maximum of 30 days, before analysis.

Venipuncture: This was carried out under aseptic condition using a syringe and needle. After sterilizing the antecubital fossa with methylated spirit, the site was allowed to air dry. A tourniquet was applied a few centimetres above the antecubital fossa to make the veins visible. Blood sample was taken using a sterilized 10ml syringe and 23G needle.

Method and instrumentation of data collection: A semi structure interviewer administered questionnaire was used to collect the socio demographic data and other relevant information from the selected pregnant women, such as bio data gestational age, haematinics etc.

Reagent and chemicals: The reagents and chemicals used for the research are Analar grade and were sourced from reliable companies.

Equipment Use for the Study

Hettich Centrifuge universal 320 centrifuge was used to spin the blood specimens. Haemogram auto-analyzer (PCE-21OE, ver. 5.10, Erma, Tokyo). Auto analyzer Spectrophotometer Micro plate reader Water bath, thermostatic with temperature regulator, thermo electron. Stadiometer model 220 (manufactured by seca Gmbh and co Germany for measurement of weight and height. Sphygmomanometer British arm cuff model for measurement of blood pressure Littman stethoscope for measurement of blood pressure and assessment of subject. Thermo cool refrigerator for storage of samples and reagents before analysis.

Anthropometric Parameters

Determination of Body Weight: Pregnant women were weighed using a mechanical lever scale which was set at zero reading. Subjects were requested to remove their shoes, heavy outer clothing and to empty their pockets to extent possible. They were asked to step on the scale platform and remain still with arms hanging naturally by the sides and forward. The weight was taken to the nearest 0.1 kilogram (Gómez-Ambrosi *et al.,* 2012)

Determination of Height: The height was determined using stadiometer. Subjects were requested to take off their footwear headgears, and excessive hair ornaments and were asked to stand with their shoulders relaxed, armed by the sides, legs straight and feet together. Measurement was taken to the nearest 1 centimetre, with the buttocks, upper back or head touching the measuring surface of the stadiometer rule. (Gómez-Ambrosi *et al.,* 2012)

Determination of Body Mass Index (BMI): The BMI was determined by dividing the weight in kilograms, by the square of the height in metres (kg/m2). (Gómez-Ambrosi *et al.,* 2012)

Blood Pressure Measurement: The blood pressure was determined at rest with the pregnant women at sitting position and the Accoson's mercury sphygmomanometer placed at the same level with the heart. The cuff of the sphygmomanometer was placed on upper arm to occlude the brachial artery. The systolic blood pressure was recorded at first audible sound while diastolic blood pressure was taken at last audible sound. (Gómez-Ambrosi *et al.,* 2012)

Analytical Method

Determination of Hematological Parameters: Haemogram auto-analyzer (PCE-21OE, ver. 5.10, Erma, Tokyo) was used to determine the Full blood count (haemoglobin, haematocrit, red cell indices, total white blood cell count and differential and platelets count)

Estimation of Serum Ferritin: Serum Ferritin concentration was estimated by a sandwich Enzyme-Linked immunosorbent Assay.

Principle: The monoclonal biotinylated antibody (anti ferritin) and serum containing native antigen (ferritin) were mixed to formed an antibody-antigen complex. At the same time biotin attached to the antibody binds to the streptavidin coated on the micro wells resulting in immobilization of the complex. Another antibody labelled with enzyme was added to form an enzyme labelled antibody-antigen-biotinylabel-antibody complex on the surface of the wells. A suitable substrate was added to produce a colour which was measured with a microplate spectrophotometer at 450nm wavelength. The enzyme activity on the well (intensity of the colour) is directly proportional to the concentration of ferritin.

Procedure

The following procedures were followed during the sample analysis.

- 25µl of specimen serum was pipetted into the assigned well.
- 100µl of the ferritin Biotin Reagent was added into each well.
- The Microplate was swirl gently for 20-30 second to mix.
- It was allowed to incubate for 30 minutes at room temperature.
- The contents of the microplate were discarded by decantation.
- 350µl of wash buffer was added.
- 100µl of the ferritin enzyme conjugate was added to each of the well.
- Then incubated again for another 30 minutes at room temperature.
- The content of the microplate was discarded by Decantation.
- 300µl of Wash buffer was added then decanted or aspirated.
- 100µl of working substrate solution was added to all wells.
- Then incubated at room temperature for 15minutes.
- 50µl of stop solution was added to each well and it was mix gently for 15-20 seconds.
- The absorbance in each well was read at 450nm (using reference wave length of 620nm

Calculations: A standard curve was plotted using absorbance for each duplicate serum reference versus the corresponding ferritin concentration in µg/L. The unknown concentrations of ferritin were determined by locating the average absorbance of the duplicates for each unknown on the vertical axis of the graph and find the intersecting point on the curve and read the concentration in µ/L from the horizontal axis of the graph.

Estimation of Serum Iron: Serum iron was estimated using Nitro-PAPS calorimetric method- Mono-reagent.

Principle: Iron is dissociated from transferrin - iron complex in weakly acid medium. The liberated iron is reduced into the bivalent form by means of ascorbic acid. Ferrous ions with Nitro- PAPS to gives a colour complex. The intensity of the colour formed is proportional to the iron concentration in the sample.

Transferrin- $(Fe^{3+})_{2+}$ e^- Ascorbic acid $2F^{2+}$ + Transferrin

Fe^{2+} + Nitro PAPS ⟶ coloured complex

Procedure: The reagent was reconstituted base on the manufacturer instructions. Two test tube were labelled as 1 (sample), 2 (blank) In each test tube One thousand microlitre (1000µl) of reagent was dispensed. Fifty microlitre (50µl) of sample was added to test tube 1 and 50µl of distilled water was added to test tube 2. This were mixed, incubated at 37^{o}C for three (3) minutes and read at 578nm against the reagent blank.

Calculations

The results were calculated as follows Serum iron (µmol/l) = A_{test} /$A_{standard}$ x $C_{standard}$

Where A= Absorbance, C= Concentration.

Estimation Of Total Iron-Binding Capacity (Tibc) And Unsaturated Iron Binding Capacity (Uibc)

The total iron binding capacity (TIBC) was determined by calorimetric method. (Gottschalk *et al.,* 2000)

Principle: Serum transferrin is saturated with an excess of Fe^{3+} and the unbound portion is precipitated with magnesium carbonate. The bound iron in the supernatant is called the total iron binding capacity (TIBC). The difference between the total iron binding capacity (TIBC) and initial Serum Iron (SI) yields the Unsaturated Iron Binding Capacity (UIBC).

Procedure: For iron saturation, 500µl of $FeCl_3$ reagent was added into a test tube with 250µl of the sample. These were mixed and incubated for 30 minutes at 25^{o}C. One spatula of magnesium carbonate was added and incubated for 60 minutes. Then it was centrifuged for ten minutes at 4000 u/min. The clear supernatant was used for determined the total iron binding capacity (TIBC). For the (TIBC), two test tube labelled 1 (sample), 2 (sample blank). One hundred microlitre 100µl was added into test tube 1 & 2, of the supernatant, then 1000µl of sample blank reagent was added to test tube 2, and 1000µl of Centronics iron Ferene reagent into test tube 1. These were allowed to mixed and incubated for 5 minutes at 37^{o} C. The absorbance of sample was measured against sample blank at 578nm wavelength.

Calculations

Unsaturated Iron binding capacity (UIBC) in µmol/l = TIBC µmol/L - serum iron µmol/L.

Determination of socioeconomic status of the perticipant

The information's from the questionnaires were used to group the pregnant women into their different socioeconomic status. Occupation, Educational level and income were the commonly used indicators of socio-economic classes (SES). However, in this study the socioeconomic classes of the pregnant women were determine by combining the score assigned to husband's occupation and the women`s Educational level developed by Olusanya et al. (2004).

Husband`s Occupation	Score
Professional	1
Middle Level	2
Unskilled	3
Woman`s Educational Status University	0
Secondary	1
Primary or no formal	2

After combining the scores assigned to husband`s occupation and women educational status.

Social class I was awarded pregnant women that score 1
Social class II was awarded to pregnant women that score 2
Social class III was awarded to pregnant women that score 3
Social class IV was awarded to pregnant women that score 4
Social class V was awarded to pregnant women that score 5
Social class I & II represent High Socioeconomic class
Social class III represent middle socioeconomic class
Social class IV & V represent low socioeconomic class

Statistical Analysis: The data obtained from the study were presented in form of tables. The results were analyzed using statistical package for social science (SPSS 20). The results were also analyzed using Microsoft excel spreadsheet and statistical software Analyzedit. Effect of socioeconomic status on serum ferritin in pregnant women was analyzed using chi-square. Statistical analysis was conducted using SPSS (version 20) software. Excel was used to compute the ratio between serum iron and total iron binding capacity. Chi square was used to compare the relation between serum level of ferritin and socio-economic status of pregnant women. Significant level was obtained at $P< 0.05$ (Gelman, 2005).

RESULTS

Socio-demographic characteristics of the participants: A total of 270 pregnant women with mean age (±standard deviation) of 26.61 (SD 5.88) years were studied. All but two were married. One hundred and twenty-nine (47.8%) of participants were in second trimester, while one hundred and forty-one (52.2%) were in third trimester and none was found at first trimester. Majority of the participants 146 (54.1%) were primary school leavers, only 19 (7%) had post-secondary school certificate and are from high socio-economic class. Two hundred and forty-one (89.3%) of the pregnant women that participated in the study were Muslim. Only 35(13%) reside in the rural area and most, 205(75.9%) of the participants were from monogamous family settings. Nearly one third of the participants 80(29.6%) were primigravida, while 44(16.3%) had more than four pregnancies. However, 170(63%) of the participants were not on haematinics during the study. Table 4.1

Socio-economic status of the participants: Most of the participants 117 (43.3%) belongs to high socio-economic class, while middle class participants were 59 (21.9%) (Table 4.2)

Haemoglobin level (Hb) of the participants*:* The mean standard deviation of haemoglobin was 10.0±1.1. Less than two third of the participants were not anaemic. (Table 4.3)

Mean corpuscular volume (MCV) of the participants: The mean standard deviation of mean corpuscular volume (MCV) was 79.3 ±6.6. More than two third, 200 (74.1%) of the participants were not anaemic. (Table 4.3)

Serum ferritin level of the participants: The mean standard deviation of serum level of ferritin was 21.6µg/L±44.2.

Table 4.1a Socio-demographic characteristics of the participants

Variables	Frequency (Percentage)
Age	
< 19	19(7.0%)
20-24	78(29.0%)
25-29	98(36.3%)
30-34	40(14.8%)
35-39	29(10.7%)
>40	6(2.2%)
Total	270(100%)
Education	
Tertiary	19(7.0%)
Secondary	105(38.9%)
Primary	146(54.1%)
Total	270 (100%)
Husband's Occupation	
Professional	130(48.1%)
Middle	45(16.7%)
Unskilled	95(35.2%)
Total	270(100%)
Marital status	
Single	2(0.7%)
Married	264(97.8%)
Divorced	4(1.5%)
Total	270(100%)
Family status	
Monogamy	205(75.9%)
Polygamy	65(24.1%)
Total	270(100%)
Gestational age	
Second trimester	129(47.8%)
Third trimester	141(52.2%)
Total	270(100%)

Table 4.1b Socio-demographic characteristics of the participants

Variables	Frequency (Percentage)
Place of Residence	
Rural	35(13%)
Urban	235(87%)
Total	270(100%)
Religion	
Islam	241(89.3%)
Christianity	29(10.7%)
Total	270(100%)
Tribe	
Hausa	195(72.2%)
Yoruba	21(7.8%)
Igbo	17(6.3%)
Others	37(13.7%)
Total	270(100%)
Parity	
Primigravida	80(29.6%)
1-2	50(18.5%)
3-4	96(35.6%)
>4	44(16.3%)
Total	270(100%)

Table 4.2. Socio-economic status of the participants

Class	Frequency	Percentage
High	117	43.3
Middle	59	21.9
Low	94	34.8
Total	270	100

Table 4.3: Haemoglobin level (Hb) of the participants

	Frequency (%)
Anaemia*	81 (30)
No-anaemia**	189 (70)
Overall	270 (100)

*haemoglobin <10.5g/l ** haemoglobin ≥10.5g/

Table 4.4. Mean corpuscular volume (MCV) of the participants

	Frequency (%)
Anaemia*	70 (25.9)
No-anaemia**	200 (74.1)
Overall	270 (100)

*mean corpuscular volume (mcv) < 80fL
**mean corpuscular volume (mcv) ≥80fL

Table 4.5. Serum ferritin level of the participants

	Frequency (%)
Anaemia*	178 (65.9)
No-anaemia**	92 (34.1)
Overall	270 (100)

*serum ferritin < 15µg/L ** serum ferritin ≥ 15µg/L

Table 4.6. Effect of socio-economic status on serum ferritin in pregnant women

Class	Anaemia status		Total
	Anaemia	No anaemia	
High	42(35.9%)	75(64.1%)	117(100%)
Middle	46(78.0%)	13(22.0%)	59(100%)
Low	90(95.7%)	4(4.3%)	94(100%)
Total	178(65.9%)	92(34.1%)	270(100%)

Chi square test of significant difference =95.21 df =4 P < 0.001

Table 4.7. Effect of socio-economic status on serum ferritin in pregnant women in second trimester gestation

Socio-economic status	Anaemia status		Total
	Anaemia	No anaemia	
High	11(14.1%)	67(85.9%)	78(100%)
Middle	12(57.1%)	9(42.9%)	21(100%)
Low	27(90.0%)	3(10.0%)	30(100%)
Total	50(38.8%)	79(61.2%)	129(100%)

chi square =95.218 df=2 P <0.001

Table 4.8. Effect of socio-economic status on serum ferritin in pregnant women in third trimester gestation

Socio-economic status	Anaemia status		Total
	Anaemia	No anaemia	
High	31(79.5%)	8(20.5%)	39(100%)
Middle	34(89.5%)	4(10.5%)	38(100%)
Low	63(98.4%)	1(1.6%)	64(100%)
Total	128(90.8%)	13(9.2%)	141(100%)

Chi square=15.287 df=2 P value<0.001

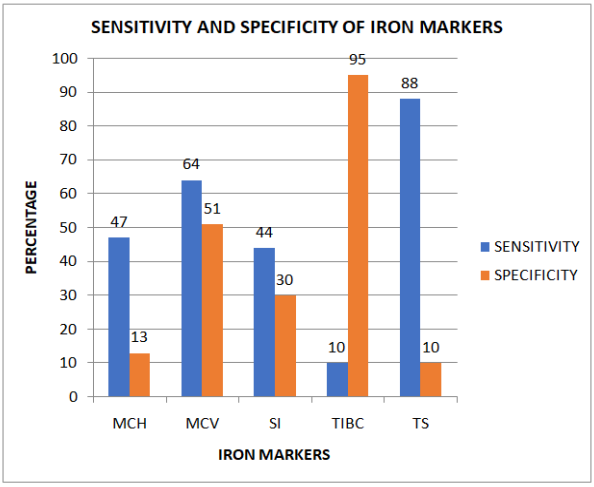

Fig. 2.0. Sensitivity and Specificity of Iron Markers

Approximately two third, 178 (65.9%) of the participants were anaemic. (Table 4.3)

Association between serum level of ferritin and socio-economic status of the participants: Low (95.7%) and middle (78.0%) socio-economic class were observed to have anaemia compared to high socio-economic class (35.9%). The difference was statistically significant (p < 0.001) (Table 4.6).

Association between serum level of ferritin and socio-economic status in second trimester: High socio-economic class 67(85.9%) were observed to have adequate iron stores, when compared to middle socio-economic class 9(42.9%) and low socio-economic class 3(10.0%) of the participants. The difference was statistically significant (p < 0.001) (table 4.7).

Association between serum level of ferritin and socio-economic status in third trimester: Low socio-economic class 63(98.4%) were observed to have inadequate iron store when compared to high social class 31(79.5%) and middle social class 34(89.5%). The difference was statistically significant (p < 0.001). (Table 4.8)

Sensitivity, specificity, positive and negative predictive values of markers for the diagnosis of iron deficiency anaemia: Serum level of ferritin was used as the reference marker, the diagnostic efficiency of iron indicators was calculated in terms of sensitivity, specificity, positive and negative predictive value. The markers include, mean corpuscular haemoglobin, mean corpuscular volume, serum iron, total iron binding capacity, and transferrin saturation.Transferrin saturation level shown to be, the best iron marker in terms of specificity, after serum ferritin.

Transferrin Saturation: Transferrin saturation, using a cut off value of <20%, its sensitivity was 88% and has a specificity of 10% for the detection of iron store in this participant. The positive predictive value of was 65% and negative Predictive value was 32%, (p=0.108).

Mean Corpuscular Volume (MCV): Mean Corpuscular Volume (MCV) is the next to transferrin saturation in terms of sensitivity. It has a sensitivity of 64%, specificity of 7% with positive and negative prediction value of 71% and 42% respectively using cut-off value of <80fL, (p=0.447).

Mean Corpuscular Hemoglobin (MCH): Mean Corpuscular Haemoglobin (MCH) has the sensitivity of 47% and specificity of 13% with positive and negative predictive value of 51% and 11% respectively using cut-off value of less than 30 Pg./cell, (p=0.135).

Serum iron: Serum iron has the sensitivity of 44% and specificity of 30% with positive and negative predictive value of 55% and 22% respectively using cut-off value of less than 8µmol/L.

Total iron binding capacity (TIBC): Total iron binding capacity (TIBC), with cutoff value greater than 93µmol/L, has the sensitivity of 10% and specificity of 95% with positive predictive value of 82% and negative predictive value of 35%.

DISCUSSION

This present study aimed to determine the serum levels of ferritin and its relationship with socio-economic status of the pregnant women.

Table 4.9. Sensitivity, Specificity, Positive Predictive value, Negative Predictive value and level of significant of Markers for the Diagnosis of Iron Deficiency Anaemia in Pregnant Women

Markers for Assessing Iron store	Anaemia status	Total	P Value	Sensitivity %	Specificity %	Predictive Value		
	Anaemia	No Anaemia					Positive %	Negative %
MCH- anaemia	85	80	165(61.1%)	0.135	47	13	51	11
no anaemia	93	12	105(38.9%)					
Total	178(65.9%)	92(34.1%)	270(100%)					
MCV- anaemia	115	45	160(59.0%)	0.447	64	51	71	42
No anaemia	63	47	110(41.0%)					
Total	178(65.9%)	92(34.1%)	270(100%)					
SI anaemia	80	64	144(53.3%)	0.929	44	30	55	22
No anaemia	98	28	126(46.7%)					
Total	178(65.9%)	92(34.1%)	270(100%)					
TIBC- anaemia	19	4	23(8.5%)	0.541	10	95	82	35
No anaemia	159	88	247(91.5%)					
Total	178(65.9%)	92(34.1%)	270(100%)					
TS- anaemia	157	82	239(88.5%)	0.108	88	10	65	32
No anaemia	21	10	31(11.5%)					
Total	178(65.9%)	92(34.1%)	270(100%)					

The prevalence of iron deficiency anaemia amongst pregnant women recruited for this study using serum ferritin level of <15µg/L was found to be 65.9%. This was significantly higher as compared to studies by Erhabor et al. (2013), Nwizu et al. (2011)and Ndukwu and Dienye (2012) with prevalence of 21.3%, 48.1% and 62.6% respectively. The lower prevalence rate of anaemia compared to these studies carried out in Sokoto, Kano and River State may be due to difference in the red cell parameters used for the determination of anaemia. Haemoglobin and haematocrit were used as marker for assessing iron deficiency anaemia, which is less sensitive compared to serum level of ferritin as described by (W.H.O., 2001). The reference ranges for haemoglobin and haematocrit were not classified based on the gestational age of the pregnancy as it can also affect the prevalence of iron deficiency anaemia in pregnancy (Pavord *et al.,* 2012).

The index study shows that anaemia was significantly associated with socio-economic status statistically (*p< 0.001*). This study revealed that, the prevalence of iron deficiency anaemia was higher among the participants of low socio-economic class. As shown in table 4.6, high socio-economic status had (35.9%) prevalence of iron deficiency anaemia, middle class had (78.0%) and lower class (95.7%). This is in agreement with a study done by Nwizu et al. (2011) in Kano State.The prevalence of anaemia in second and third trimester among the different socio-economic classes was similar. Table 4.7 and 4.8. The high prevalence of iron deficiency anaemia in this study may be as a result of poverty and malnutrition. The prevalence of iron deficiency anaemia (90.8%) among pregnant women was significantly higher in third trimester gestation than those in their second trimester (38.8%).This finding is in line with one study carried out in Indian by Raza et al. (2011), which revealed that, pregnant women that booked at third trimester were at risk of developing iron deficiency anaemia. Assessing iron status using haemoglobin concentration is not reliable in pregnancy due to physiological changes that occurred in plasma volume; moreover, it is nonspecific for iron (Raza *et al.,* 2011). Ferritin is considered to be the most accurate method available to determine iron store provided there is no infection or inflammation (Cook and Finch, 1979). Therefore, serum level of ferritin was used as 'reference standard' against which other haematological and biochemical parameters were compared. Percentage Transferrin saturation of less than 20% was considered as iron deficiency anaemia and the present study shows that the transferrin saturation is the most sensitive, with a sensitivity of 88%. This makes transferrin saturation the best tool for identifying iron deficiency anaemia in pregnancy when compared with other markers like mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), serum iron and total iron binding capacity (TIBC). This is in agreement with a study carried out in Canada by Mazza et al. (1978). The study showed the sensitivity of transferrin saturation to be the highest among other markers used in assessing iron deficiency anaemia. Transferrin saturation is the ratio of serum Iron to total Iron binding capacity. The specificity was very low in this study which also corresponds to other studies by Mazza et al. (1978), Kis and Carnes (1998). The sensitivity of transferrin saturation and other Iron markers tend to be decreasing as gestational age advances. This is in line, as haemodilution affects the concentration of those markers (Cook *et al.,* 1974). The next marker to transferrin saturation in terms of sensitivity is the mean corpuscular volume (MCV). A decrease in mean corpuscular volume of less than 80fL indicates a decrease in haemoglobin synthesis. In this present study the sensitivity of mean corpuscular (MCV) was (64%), this is almost similar with a study carried out by Den Broek (1998) that showed the sensitivity of mean corpuscular volume (MCV) to be 58%. Serum iron is not widely used for assessing iron status because of diurnal variation, and recently ingested iron can also affect it (Burtis and Bruns, 2014). In this present study total iron binding capacity (TIBC) had the lowest sensitivity of (10%) this correspond with a research carried out by Den Broek (1998). Total iron binding capacity (TIBC) has the highest ability to detect correctly those without anaemia that is specificity of (97.4%). If anaemia in pregnancy is as result of iron deficiency and there are no features of infection or malignancy, determination of iron status using serum level of ferritin is more superior over transferrin saturation, mean corpuscular volume, serum iron concentration, haemoglobin, haematocrit, as these markers are often within normal reference value in mild anaemia (Mazza *et al.,* 1978).

Conclusions and Recommendation

Conclusions

The findings of this particular study showed that, socio-economic status greatly influence iron stores of the pregnant women.

Also, the prevalence of iron deficiency anaemia is on the increasing side amongst pregnant women attending antenatal care at Specialist Hospital Sokoto. There is need to include serum level of ferritin in the routine antenatal investigation for the assessment of iron status, as it reflects the true iron store. This will help making early diagnosis to prevent the complications of iron deficiency anaemia. There is also need to remind the government, on implementation of policies that has direct benefit in the improvement of socio-economic status in people living in the rural area, like poverty eradication, easy accessibility to health care centre, provision subsidized drugs.

Recommendation

There is need to include serum level of ferritin in routine investigation at booking, in our antenatal clinic, this will assist us in identifying iron deficiency at early stage. There is also need to improve socioeconomic factors associated with anaemia such as education and employment.

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