ASSESSMENT OF BIOCHEMICAL MARKERS OF IRON AND SOME HAEMATOLOGICAL PARAMETERS ON BLOOD DONORS IN ENUGU STATE

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A PROJECT PRESENTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE (M.SC) IN DEPARTMENT OF MEDICAL LABORATORY SCIENCES,

FACULTY OF HEALTH SCIENCE AND TECHNOLOGY,
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DECEMBER, 2014

DEDICATION

This work is dedicated to the Almighty God who gives knowledge and wisdom

ACKNOWLEDGEMENT

I whish to express my profound gratitude to GOD Almighty who has seen me through the ups and downs in the course of this study.

My love goes to my husband, Charles who encouraged and supported me both financially and spiritually through out the course of this study.

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I must not fail to make reference to my dear friend Dr. Enyiuche Doris Ezigbo (Ph.D). Who was also a co- supervisor, for making out time to go through this project and do necessary correction.

I am grateful to the Ethical committee of the University of Teaching Hospital, Ituku Ozalla, for giving me the authority to carry out my research work and also to the staff of Blood Transfusion unit UNTH, for their immense help in collection and assessment of the samples. Also am deeply indebted to the director and staff of National blood transfusion services, Enugu, Medichem medical laboratories,and Cynsoki medical laboratories, for their contribution and assistance in collection of samples. I will not fail to recognize the contribution of Life Care Specialist medical laboratories for their assistance in purchasing of the reagents and assessment of samples.

I sincerely do appreciate everyone who committed their time and effort to see to the success of this work. I say a very big thank you to you all. God bless you.

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ABSTRACT

Iron deficiency is one of the most common of the nutritional deficiencies. The eventual consequence of iron deficiency is iron deficiency anemia; where the body stores of iron have been depleted and the body is unable to maintain level of hemoglobin in the blood. Blood donation leads to a substantial iron loss, as about 0.5mg iron is lost per each milliliter of blood donated. It is evident that haemoglobin level alone is inadequate for detecting blood donors with iron deficiency without anaemia. Studies have also shown that hemoglobin level may not correlate with iron status. The aim of this study, however was to assess the biochemical indices of iron and some haematological parameters amongst donors in Enugu State. Two hundred apparently healthy male blood donors from National blood Transfusion centre Enugu, University of Nigeria Teaching hospital and two private blood banks were used for the study. Forty apparently healthy non-donors were used as controls. Before starting the study, approval of the institutional ethical committee was obtained and informed consent was also obtained from the donors and controls to enable them participate effectively in the study. The donors were divided into 6 groups according to number of previous donations within last 3 years. The age range was between 19-50 years. Results showed that level of Hb, PCV, and iron status parameters were significantly decreased among the regular donors compared to controls. The mean ±2SD in ferritin level showed a significant decrease across the groups ($P<0.0001$) with 21.46±6.76ng/ml, 16.11±16.06ng/ml, 6.76± 5.54ng/ml for subjects in groups iv, v, and vi in 3yrs respectively compared to 52.67±40.47ng/ml, and 51.20±23.25ng/ml for subjects in group ii and iii in 3yrs. The serum iron concentration was significantly higher with group i (control), group ii and group iii donors: 94.17±27.66µg/ml, 113.8±71.20µg/ml, 83.71±10.84µg/ml respectively compared to donors in groups iv, v, and vi: 72.73± 10.98µg/ml, 70.00± 11.62µg/ml, 65.83± 15.57µg/ml respectively . The TIBC increased significantly ($P<0.000$) across the groups with mean values: 371.2±76.14µg/ml, 455.4±11.9µg/ml and 481.0± 98.62µg/ml for donors in group iv, v and group vi respectively in 3yrs. The mean ± 2SD for transferrin saturation showed a significant decrease ($P<0.0001$) with donors in group iv, v and vi having 20.50± 5.66%, 23.60± 10.74%, and 20.64± 8.22% respectively compared to group i(control), group ii and iii donors: 31.38± 10.47%, 33.47±15.4%, 34.47± 12.8% respectively. The Hb and PCV of the subjects were significantly reduced ($P<0.0001$) with the donors in group iv, v and vi having 14.73g/dl± 3.28, 13.64g/dl±0.76, 13.78g/dl± 0.63 respectively in 3yrs compared to group i(control), group ii and group iii donors. There was no significant difference in the MCHC, WBC Total, differentials and across the groups. Also there was no significant difference in the iron and haematological parameters of group i(controls) compared to the donors in group ii and group iii in 3 years. The study observed that Haemoglobin, Packed cell volume, Serum iron, Serum ferritin, Transferrin saturation, TIBC, were significantly affected in the regular blood donors especially those who have had more than six donations in 3 years when compared with apparently healthy controls.

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Iron deficiency (Sideropenia or hypoferremia) is one of the most common of the nutritional deficiencies. Iron is present in all cells in the human body, and has several vital functions, example includes as a carrier of oxygen to the tissues from the lungs in the form of haemoglobin, as a transport medium for electron within the cells in the form of cytochrome and as an integral part of enzyme reactions in various tissues. Too little iron can interfere with these vital functions and lead to morbidity and death.

The eventual consequence of iron deficiency is iron deficiency anaemia where the body's stores of iron have been depleted and the body is unable to maintain level of haemoglobin in the blood. Total body iron averages approximately 3.3g in men and 2.3g in women. In blood, plasma iron is carried tightly bound to the protein transferrin. When loss of iron is not sufficiently compensated by adequate intake of iron from the diet; a state of iron deficiency develops overtime as dietary iron is important in oxygen transport and the synthesis of iron. When this state is not corrected, it leads to iron deficiency anaemia (Wintergerst *et al,* 2007).

Most well nourished people in industrialized countries have 4 to 5 grams of iron in their bodies. Of this about 2.5g is contained in the hemoglobin needed to carry oxygen through the blood and most of the rest (approximately 2g in adult men, and somewhat less in women of child bearing age) is contained in ferritin complexes that are present in all cells but most common in bone marrow, liver and spleen.

Iron deficiency is a reduced content of total body iron. Iron deficiency ranges from depleted iron stores without functional or health impairment to iron deficiency with anaemia which affects the functioning of several organs/system (Akman *et al,* 2004). One study suggests that, in otherwise healthy people, ferritin levels of <22mg/L could be used to define clinically relevant iron storage depletion (this includes the pre-anaemic stages of iron deficiency) (Suominen *et al*, 1998). Iron deficiency anaemia is sufficient to reduce erythropoiesis and therefore the haemoglobin level falls. However problems related to iron depletion can develop before this stage.

There is less information on iron depletion with normal haemoglobin level. This is known as non anaemic iron deficiency. Non anaemic iron deficiency is sometimes termed latent iron deficiency or depleted iron stores (Lichtman *et al* 2005). Iron depletion may be three times as common as iron deficiency anaemia which has a prevalence of 2-5% of adult men and post menopausal women in the developed world, but more common in the developing world (Suominen *et al,* 1998). Iron stores are considered depleted when serum ferritin value are between 12-14/µg/l, reduced when between 15-30µg/l, normal when values are between 31-300µg/l (Simon *et al,* 1981). Blood donation is one of the aetiology of non anaemic iron deficiency. However there may be no symptoms until significant anaemia develops (Rasvik *et al,* 2009).

The impact of blood donation on iron Status has been studied since the 1970's. Blood donation results in a substantial (200 - 250mg) loss of iron at each collection procedure, during which up to 425 - 475ml of whole blood are withdrawn, and subsequent mobilization of iron from body stores. Chronic iron deficiency is a well recognized complication of regular blood donation. An increase in the frequency of

blood donation among our donor population is liable to result in excessive iron loss and development of iron deficiency anaemia. It is well known that iron deficiency anaemia is the last stage of iron deficiency and it is evident that haemoglobin levels alone are inadequate for detecting blood donors with iron deficiency without anaemia. Studies have also shown that haemoglobin level may not correlate with iron Status (Rangaragan Sunad, 2007).

Regular blood donation may be an important cause of iron loss and chronic iron deficiency. Each 1.0ml of blood contains approximately 0.5mg of iron. A unit of donated blood therefore contains approximately, 250mg of iron in men and 213mg in women. Iron stores are approximately 30% lower in female donors than in male donors who give one unit of blood yearly (Morashikin *et al*, 2006).

Iron deficiency has been observed in long term blood donors in the majority of blood banks. A healthy individual can donate blood up to three times a year at 4 months interval as iron stores can be depleted if blood is donated more frequently (Morashikin *et al,* 2006). After a single donation, a person needs sapproximately 3 months to replenish iron stores. Some experts believe that frequent bleeding even with iron supplementation is not justified and that the maximum annual rate of donation should be twice for men and once for women.

No transfusion service can survive without blood donors. The well being and health of the blood donors is of prime importance for the medical profession. A lot has been discovered and written about protection of the recipients from the potential hazards of blood transfusion. A lot of money is being spent for the screening of donors for protection of recipient, but very little attention is given to the health status of donors. The main reason is the fear of losing the donors and the result is that many blood donors presents more than three times a year. In the

absence of a national blood bank service to regulate the practice of blood donation, the safety of blood supplies and that of donors is source of concern.

In the majority of blood banks haemoglobin and/or haematocrit measurement are used as screening test for the ability to donate blood even though iron stores may be depleted in donors with haemoglobin values above the arbitrarily defined limit for anaemia (Baynes, 1994). A recent pilot study by Nubilla *et al* (2014), suggested that the screening procedure of potential blood donors at aTertiary hospital, Enugu is fair, though not complete and hence needs to be standardized. They recommended complete blood count and blood film as one of the parameters to be assayed on blood donors in order to improve the screening of blood donors and ensure safe blood transfusion. However, the use of these parameters has been reported to have poor sensitivity in the detection of early stages of iron deficiency (Agba *et al,* 1989). In order to fulfill the global national drive to recruit and retain regular repeat voluntary blood donors, the iron status of the donors need to be identified (Cancado *et al,* 2001).

JUSTIFICATION

Regular blood donation can lead to preclinical iron deficiency as well as iron deficiency anaemia if necessary steps for iron supplementation is not taken. This is because some of these donors do not adhere strictly to the acceptable standards of frequency of donation. There is a need to increase the voluntary blood donation for safe blood supply. However, there is paucity of data in this part of the country regarding impact of regular blood donation on iron status of donors. The present study was therefore designed to assess using a combination of haemoglobin and iron status parameters, the development of anaemia and assessment of iron deficiency anaemia amongst blood donors in this area of Nigeria.

1.2 AIM

To assess the biochemical markers of iron and some haematological parameters on blood donors in Enugu State

1.3 OBJECTIVES

1 To determine the level of serum ferritin, serum iron, transferrin saturation and total iron binding capacity among blood donors in Enugu state.
2. To determine the iron status of donors in relation to the frequency of donations.
3. To determine some haematological parameters of donors in relation to the frequency of donations.

CHAPTER TWO

2.1 LITERATURE REVIEW

Iron is present in the oxygen carrying protein of the red blood cell hemoglobin. Iron turnover is also dominated by the synthesis and breakdown of hemoglobin. Everyday about 30mg of iron are used to make new hemoglobin and most of this is obtained from the breakdown of old red cells. The body iron content is maintained by variation in the amount of iron absorbed. In women, menstruation and childbirth increases iron losses to about 2mg/dl (Flemming and Bacon, 2005) Iron absorption may not increase sufficiently to compensate for these iron losses and this may eventually lead to the development of iron deficiency anemia. In most men and postmenopausal women, there is some storage iron know as ferritin or its insoluble form, haemosiderin, which is available for haem synthesis if necessary. Many young women and children have little or no storage iron (cook *et al,* 2003). One of the most prevalent disorders of man is the dietary deficiency of iron and resulting anaemia. Therefore the assays of iron, total iron binding capacity and other assessment of iron compounds in the body are clinically significant. Iron storage compounds in the body include hemoglobin, hemosiderin, myoglobulin and the cytochromes.

2.2 DIETARY IRON ABSORPTION

Iron absorption depends on the amount of iron in the diet, its bioavaliability and the bodyôs need for iron. The iron available in the diet is absorbed in a small portion only. This amount also depends on the interaction of food, drugs and abnormal component of diet. A normal western diet provides approximately 15mg of iron daily. Of that iron, digestion within the gut lumen releases about half in a

soluble form, from which only about 1mg (5 ï 10% of dietary iron) is transferred to the portal blood in a healthy adult male (Alvarez *et al,* 2000). The absorption of dietary iron is a variable and dynamic process. The amount of iron absorbed compared to the amount ingested is typically low, but may range from 5% - 35% depending on circumstances and type of iron. Non haem iron is released from protein complexes by acid and proteolytic enzymes in the stomach and small intestine. It is maximally absorbed from the duodenum and less well from the jejunum (Mckie *et al,* 2011). Like most mineral nutrients, the majority of the iron absorbed from digested food or supplements is absorbed in the duodenum by enterocytes of the duodenal lining. These cells have special molecule that allow them to move into the body. To be absorbed directly, iron can be absorbed as part of a protein such as heme protein or must be in its ferrous ($fe^{2+)}$ form . A ferric reductase enzyme on the enterocytes brushboarder, Dcytb (Duodenal cytochrome B) reduces ferric fe^{3+} to fe^{2+}. A protein called divalent metal transporter (DMTI), which transports all kinds of divalent metals into the body, then transports the iron across the enterocytes cell membrane and into the cell(Andrews, 2000). These intestinal lining cells can then either store the iron as ferritin, which is accomplished by fe^{3+}, binding to apo- ferritin in which case the iron will leave the body when the cell dies and is sloughed off into feces or the cell can move it into the body using a protein called ferroportin. The body regulates iron level by regulating each of these steps. For instance, cells produce more Dcytb, DMT1 and ferroportin in response to iron deficiency anaemia. While Dcytb is unique to iron transport across the duodenum, ferroportin is distributed throughout the body on all cells which store iron. Thus regulation of ferroportin is the bodyôs main way of regulating the amount of iron in circulation.

2.3 IRON STORAGE

All cells require iron for the systhesis of protein but also have the ability to store excess iron for the synthesis of protein. Iron is stored, mostly in the liver, as ferritin or haemosiderin. Ferritin is a protein with a capacity of about 4500 iron (III) ions per protein molecule. This is the major form of iron storage. If the capacity for storage of iron in ferritin is exceeded, a complex of iron with phosphate and hydroxide forms. This is called hemosiderin. It is physiologically available.

As the body burden of iron increases beyond normal levels, excess haemosiderin is deposited in the liver and heart. This can reach the point that the function of these organ is impaired, and death ensures (Frey *et al*, 1995).

Most well nourished people in industrialized countries have 4 to 5 grams of iron in their bodies. Of this, about 2.5g is contained in the hemoglobin needed to carry oxygen through the blood, and most of the rest is contained in ferritin complexes that are present in all cells, but most common in bone marrow, liver and spleen. The liverôs stores of ferritin are the primary physiologic source of reserve iron in the body.The reserves of iron in adults in industrialized countries tend to be lower in children and women of child bearing 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 (Harrison, 1990).

Iron deficiency first affects the storage iron in the body, and depletion of these stores is thought to be relatively non symptomatic, although some vague and non specific symptoms have been associated with it. Since so much iron is 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 intercellular processes like electron transport.

Macrophages of the reticuloendothelial system store iron as part of the process of the breaking down and processing hemoglobin from Engu lfed red blood cells (Rockey and cello, 1993)

2.4 HOW THE BODY GETS ITS IRON

Most of the iron in the body is hoarded and recycled by the reticulo endothelial system, which breaks down aged red blood cells. However, people lose a small but steady amount by gastrointestinal blood loss, sweating and by she dding cells of the skin and mucosal lining of the gastrointestinal tract. People with gastrointestinal parasitic infections, more commonly found in developing countries, often lose more. This steady loss means that people must continue to absorb iron. They do so via a tightly regulated process that under normal circumstance protects against iron overload (Thei, 1987).

2.5 ABSORBING IRON FROM THE DIET

Like most mineral nutrients, the majority of the iron absorbed from digested food or supplements is absorbed in the duodenum by enterocytes of the duodenal lining. These cells have special molecule that allow them to move into the body. To be absorbed directly, iron can be absorbed as part of a protein such as heme protein or must be in its ferrous ($fe^{2+)}$ form . A ferric reductase enzyme on the enterocytes brushboarder, Dcytb (Duodenal cytochrome B) reduces ferric fe^{3+} to fe^{2+}. A protein called divalent metal transporter 1(DMTI), which transports all kinds of divalent metals into the body, then transports the iron across the enterocytes cell membrane and into the cell.(Andrews, 2000). These intestinal lining cells can then

either store the iron as ferritin, which is accomplished by fe^{3+}, binding to apo-ferritin in which case the iron will leave the body when the cell dies and is sloughed off into feces or the cell can move it into the body using a protein called ferroportin. The body regulates iron level by regulating each of these steps. For instance, cells produce more Dcytb, DMT1 and ferroportin in response to iron deficiency anaemia. While Dcytb is unique to iron transport across the duodenum, ferroportin is distributed throughout the body on all cells which store iron. Thus regulation of ferroportin is the bodyôs main way of regulating the amount of iron in circulation.

2.6 REASONS FOR IRON DEFICIENCY

Functional or actual 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, such as calcium, phytates and tannins.
- Inability to absorb iron because of damage to the intestinal lining e.g surgery involving duodenum, crohnôs or celiac sprue.
- Inflammation

In iron deficiency, transferrin receptor production will increase and ferritin production will decrease (Banerjee *et al,* 1986).

2.7 REGULATION BY LOCATION

When body levels of iron are too low hepcidin in the plasma is decreased. This allows an increase in ferroportin activity, stimulating iron uptake in the digestive system. When there is an iron suplus more hepcidin is released by the liver thus blocking additional ferroportin activity, resulting in less iron uptake.

Individual cells, an iron deficiency causes responsive element binding protein to iron responsive elements on MRNA for transferrin receptors, resulting in increased production of transferrin receptors. These receptors increase binding of transferrin to cells and therefore stimulating iron uptake. Normal iron status implies a level of erythropoiesis that is not limited by the supply of iron and the presence of a small reserve of ñstorage ironò to cope with normal physiological needs. The ability to survive the acute loss of blood (iron) that may result from injury is also an advantage. The limits of normal are difficult to define and some scientists argue that physiological normality is the presence of only a minimal amount of storage iron but the extremes of iron deficiency anemia and haemochromatosis are well understood.

Iron deficiency should be suspected in hypochromic, microcytic, anemia but in the early stages of iron deficiency red cells may be normocytic and normochromic. It is the most common nutritional disorder affecting at least one third of worldôs population. Though anemia is a common manifestation of iron deficiency, other effects of iron deficiency on various tissues, organs and systems are usually under recognized. Impaired brain development and cognitive behavioral and psychomotor impairment are most worrisome manifestation of iron deficiency.

Studies have demonstrated that some of these changes occurring during period of brain growth spurt (<2years) may be irreversible. Iron deficiency is clearly seen in men after the first blood donation and is more intense in women; as their previous reserves are lower (Hemandez *et al*, 1994). A large proportion of both female and male frequent blood donors have iron depletion. Donation intensity, sex and menstrual status, weight and age are important independent predictors of iron deficiency.

Reducing the frequency of blood donation is likely to reduce the prevalence of iron deficiency among blood donors, as might implement routine iron supplement (Brittenham, *et al* 2001). In a large Danish study, the prevalence of depleted iron stores was found to be higher in donors than in people who did not donate blood.

A significant relationship was seen between the mean of serum ferritin and instances of blood donation and this suggests estimation of serum ferritin to check preclinical iron deficiency among donors (Mittal *et al*, 2006).

Djalali, *et al* (2006), in their study showed that the level of hemoglobin, heamatocrit and iron status indicies were all significantly decreased in the subjects than in controls and a gradual but significant decrease in iron status indices in each time of blood donation. According to Vilsu *et al*, (2008), significant anemia and iron deficiency was observed in >50 categories of regular voluntary blood donors. In a study of serum ferritin levels among male blood donors in Malaysia by Norashikin *et al* (2006), the serum ferritin levels were found to be significantly lower among the regular donors compared to first time donors. The serum ferritin levels gradually decrease according to the number of donations and there was a significant correlation between frequency of donation and serum ferritin level and also hemoglobin level. According to Vilsu *et al*, (2008), significant anemia and

iron deficiency was observed in >50 categories of regular voluntary blood donors. In that same study, there was no significant difference in the iron profile of vegetarians and non-vegetarians subjects or controls and the donors donating < 20 times.

Iron status of 592 blood donors was analyzed and it was observed that the reduction in iron stores was related to the frequency rather than the total number of donations. 8% had reduced iron stores but this was not related to blood donation, iron supplement and sex of the donors. The frequency of donations per year was more predictive of decreased iron stores rather than the number of life time donations (Mittal *et al*, 2006).

In a similar study in Iran by Javadzadeh *et al* (2003), there was a significant relationship between the last donation period, iron deficiency and iron deficiency anemia among donors, therefore there was a higher percentage of iron deficiency and iron deficiency anemia among donors with record of 12 months ago and less in comparison to the group with longer than 13months.

In a large danish study, the prevalence of depleted iron stores was found to be higher in donors than in people who did not donate blood (Milman and Kirchoft, 1991). Futher more in the study by Badar *et al (*2002), 26% of regular blood donors, had low ferritin levels and 12% were found to be anemic.

Oluboyede *et al* 1983, evaluated serum ferritin levels and haematological parameters in a Nigerian population and observed that haematological values fell within the reported range for healthy population in other parts of the world but the mean serum ferritin value in the male population was 72.4ng/ml compared with the female value of 34.2ng/ml.

In Nigeria, Usanga(1990), observed that the mean ferritin concentration of 64.75ng/ml ± 4.6 in normal males was significantly higher than the mean value of 49.19ng/ml among blood donors suggesting that some blood donors may have prelatent or latent iron deficiency at the time of donation and become iron deficient after blood donation.

In contrast, in a recent study of blood donors in Calarbar, Nigeria, Akpotuzor *et al* (2008), observed no difference in biochemical iron parameters between male donors and healthy controls. Although in that study donors were not separated into several categories.

Ealier study in India has reported that 73.8% of professional blood donors are anaemic (Mittal *et al*, 2006). Also there was no coincidence between low haemoglobin and low serum ferritin when haemoglobin was within normal range (Halmorsen *et al* 1990). According to Krishna and Taylor, 2002, ferritin levels tended to be lower in younger people, females and those with more intensive blood donation history.

A study from the United States of America, suggested that up to 8% and 23% of male and female donors respectively may be iron deficient. According to Norashikin *et al* (2006), though reapeted blood donation might diminish iron status, it could be safe to donate 2-3 times a year without an appreciable incidence of iron deficiency, provided that the predonation Hb and ferritin values are > 14.7g/dl and 58.9µg/l respectively.

In a recent study in Iran by Vahid youse fine Jad *et al* (2010), there was a significant relationship between the last donation period, iron deficiency and iron deficiency anaemia among blood donors . Iron deficiency anemia was significantly

higher among donors with more than ten instances of donation. Also a significant relation was seen between the mean of serum ferritin and instances of blood donation and this suggests estimation of serum ferritin to check preclinical iron deficiency among donors (Badar *et al,* 2002).

2.8 HAEM IRON

Iron deficiency is the most common cause of anaemia and usually results from blood loss. Symptoms are usually nonspecific. Red blood cells tend to be microcytic and hypochromic and iron stores are low as shown by low serum ferritin and low serum iron levels with high serum total iron binding capacity.

Iron is distributed in active metabolic and strong pools. Total body iron is about 3.5g in healthy men and 2.5g in women. The difference relates to womenôs smaller body size, lower androgen levels and dearth of stored iron because of iron loss due to menses and pregnancy. The distribution of body iron in average man is Hb, 2100mg, ferritin 700mg (in cells and plasma), hemosiderin; 300mg (in cells), myglobin; 200mg, tissue (heme and non heme) enzymes, 150mg, and transport-iron compartment; 3mg. Iron is a critical micronutrient, and iron derived from heme contributes a large proportion of the total iron absorbed in a typical western diet. Haem iron is absorbed by different mechanism than non-heme iron, but despite considerable study over many years these mechanism remain poorly understood. There are two prevailing hypothesis of heme absorption namely receptor mediated endocytosis of heme, and direct transport into the intestinal enterocytes by recently discovered heme transporters. A specific emphasis is placed on the questions surrounding the site of heme catabolism and the identity of the enzyme that performs this task (Lieu *et al*, 2001).

The importance of dietary iron and heme iron is a vitally important element in biological terms. Iron is a transition metal with the ability to readily accept and donate electrons allowing it to function as an oxidant or reductant in a large number of biochemical reactions. In mammals, iron is notably required for oxygen transport as a component of haemoglobin, DNA synthesis as a component of ribonucleotide reductase, and as an electron acceptor/donor with cytochromes that are essential for energy transduction. Currently, iron deficiency is the most common diet related health problem in the world and the effects on human health are wide ranging(Gibson and Ashwell, 2003).

Heme is a biologically important iron containing compound and a key source of dietary iron. Currently, the importance of heme iron in the diet cannot be under estimated. Studies estimate that in western societies, iron derived from heme sources such as myoglobin and hemoglobin make up two-thirds of the average personôs total iron stores despite only constituting one-third of the iron that is actually ingested (Narasinga, 1981). This likely explains why vegetarians are more prone to iron deficiency than those who regularly consume red meat. The relative importance of dietary heme is attributable to its high bioavailability compared with non-heme iron in the predominantly alkaline conditions found in the lumen of the small intestine. In aqueous solutions at or above PH 7.0, non-heme iron is present as fe(ii) and fe(iii). Fe(ii) readily oxidizes to fe(iii) which precipitates from solution as ferric hydroxide or forms soluble hydroxyl-iron dimers which are not directly available for absorption (Bjorn-Rasmussen *et al*,1974). Many dietary components (particularly humic substances such as tannins and phytate) can chelate iron making it non-bioavailable, while only select reductants in the diet (such as ascorbate) can act as solubilizing agents (Carpenter *et al*, 2002)

2.9 A possible role for DMTI (Divalent Metal Transporter)

The uptake of non-heme iron by the enterocytes occurs through the function of DMTI. DMTI is also capable of transporting other divalent cations and is responsible for cellular iron acquisition during the transferrin receptor cycle.

DMTI functions as an fe(ii) / proton symporter and is highly expressed in the duodenum during iron deficiency . This is consistent with the duodenum being the principal site of iron absorption and the least alkaline section of the small intestine due to the close proximity to acidic gastric secretions. Ultimately, fe(iii) is also transported by DMTI after reduction to fe(ii) by DcytB, although the physiological significance of this pathway is the subject of continued debate.

Iron is absorbed in the duodenum and upper jejunum. Absorption of iron is determined by the type of iron molecules and by what other substances are ingested. Iron absorption is best when food contains heme iron (meat).Dietary non-heme iron must be reduced to the ferrous state and released from food binders by gastric secretions. Non heme iron absorption is reduced by other food items (eg, vegetable fibre, phytates and polyphenols, tea tannates, including phospho proteins, bran) and certain antibiotic (eg tetracycline). Ascorbic acid is the only common food element known to increase non heme iron absorption. There are three sequential stages through which impairment of iron supply can lead to established or frank iron deficiency anaemia(Carpenter *et al*, 1992).

1. **Depletion of Iron Stores**

Iron depletion may be three times as common as iron deficiency anaemia which has a prevalence of 2-5% of adult men and post menopausal women in the world. Iron depletion is more common in the developing world (suomminen *et al*,

1998).This occurs in the state of inbalance of serum iron supply. If adequate amount of iron for the synthesis of haemoglobin and other dependent compounds in the body is not readily available in serum, iron is released from the stores to control this situation. In this stage (a)iron absorption is high (b) Iron store is reduced (c) Serum iron level may still be normal (d) Serum ferritin is reduced.

2. Iron Deficiency Erythropoiesis

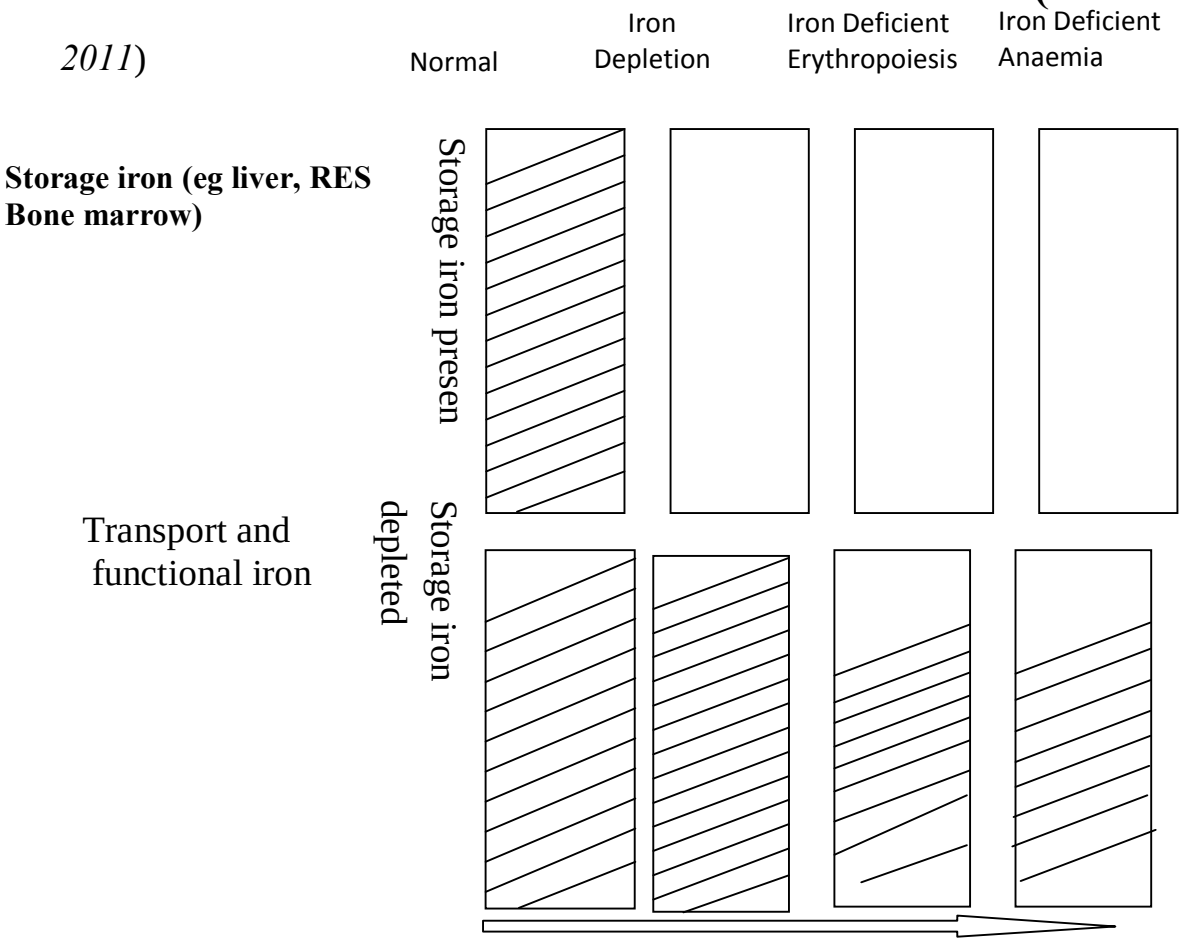
If iron therapy is administered in stage one there may be restoration of iron stores and everything will become normal. Otherwise as iron continues to be depleted to the extent of serum ferritin falling below 15µg/l, iron deficient erythropoiesis begins to occur. In this sage (a) there is increased concentration of transferrin receptor and (b) red cell protoporphyrin is increased (c) MCV and MCHC may still be within the refence range. Erythropoietin (EPO) is a gycoprotein hormones produced in the kidney that acts as a forunner for red blood cell in the bone marrow and stimulates the manufacture of hemoglobin (Delarme etal, 1992). A change in the red blood cells will affect the release of EPO. Erythropeietin therefore is part of the finely tunned feedback circuit that control red blood cell levels. The distribution of body iron stores show the importance of iron to red cell production. Normally about 70% of iron is formed in the circulating erythrocytes, approximately 20% is stored as ferritin in the liver. When iron is absorbed, about 80% is delivered to the bone marrow where a feedback mechanism exists such that iron absorption increases when more red cells are produced by the bone marrow (Adamson,1994). Erythropoiesis involves the close interaction of iron and erythropoietin. Iron is the fuel for the production of new red blood cells. When the two are coupled, red cell production moves briskly and efficiently. Even when both components are available they must be coordinately delivered to the bone marrow

for proper action. For instance, if iron arrives on the scene 6hrs after erythropoietin reaches the bone marrow, red cell production will be suboptimal. The erythropoietin would have spent itself on cells that where unable to respond. Little or none of the erythropoietin would be left when the iron finally arrives. Therefore if erythropoietin is present without sufficient iron, there is insufficiency fuel for red cell production (Hotta *et al* 1991).

3. Established Iron deficiency Anaemia

Again if stage two is uncontrolled the situation may become worst to the extent that frank iron deficiency anaemia develops. In this stage the red blood cells show these features; Microcytosis, Hypochromasia, Serum ferritin is <15µg/l, Transterrin saturation is < 15% , Hemoglobin level is <12gdl. Iron deficiency anaemia is a common anaemia caused by insufficient diet intake and absorption of iron, or iron loss from bleeding which can originate from a range of sources. Iron deficiency anaemia can result if the body does not make enough red blood cells or if bleeding caused loss of red blood cells more quickly than they can be replaced. Anaemia is one result of advanced stage iron deficiency. When the body has sufficient iron to meet its needs (functional iron), the remainder is stored for later use in all cells and spleen. These stores are called ferritin complexes and are part of the human iron metabolism systems.

2.9.1 SPECTRUM OF IRON DEFICIENCY (Goddard *et al, 2011*)



PROGRESSIVE IRON DEPLETION

Serum Ferritin(µg/l)	60	<15	<15	< 15
Transferrin saturation (%)	35	<35	<15	<15
Hemoglobin(g/l)- Female	>120	>120	>120	<120
Hemoglobin (g/l)- Male	>130	>130	>130	<130

Spectrum of iron deficiency

The iron content of the human body may be divided into three classes: iron in storage, iron in use and iron in transport. Serum iron assays measure transport iron bound to the protein transferrin. Increase in serum iron level may indicate increased erythrocyte destruction, decreased erythrocyte formation, increased absorption or defects in storage capabilities. Decrease in serum iron level may indicate iron deficiency or inability to retrieve storage iron.Iron deficiency results from extensive negative iron balance, culminating in decreased or exhausted iron stores. Sequential stages are normal iron status to iron depletion (depletion of storage iron) to iron deficient erythropeiosis (red cell production is affected but hemoglobin still within normal level) and finally iron deficiency anaemia.

2.9.2 NON ANAEMIC IRON DEFICIENCY

Iron deficiency without anaemia is three times as common as iron deficiency anaemia (IDA). Studies in females, adolescents and youth have shown that iron deficiency without anaemia has a number of clinical consequences similar to IDA, including fatigue, impaired concentration and decrease work productivity. There is a lack of research regarding the effects of iron deficiency without anaemia in males (Goddard *et al*, 2011).

2.9.3 FERRITIN

Ferritin is a ubiquitous intracellular protein that stores iron and releases it in a controlled fashion. In human, it acts as a buffer against iron deficiency and iron over load (langlois *et al*,2004). Ferritin is found in most tissues as a cytosolic protein, but small amounts are secreted into the serum where it functions as an iron carrier. In most tissues ferritin is a major iron- storage protein. High concentration

of ferritin are found in the cytoplasm of reticuloendothelial system, the liver, spleen and bone marrow. Serum ferritin level can be measured routinely and are partially useful in the early detection of iron deficiency anaemia in apparently healthy people. Serum ferritin measurement is also clically significant in the monitoring of iron status of pregnant women, blood donors and renal dialysis patients. Hence serum ferritin is used as a diagnostic test for iron deficiency anaemia. Ferritin that is not combined with iron is called apoferritin. Ferritin serves to store iron in a non-toxic form to deposit it in a safer form, and to transport it to areas where it is required (Rachel and Regina,2008). As ferritin accumulates within cells of the reticulo endothelial system, protein aggregates are formed as hemosiderin. Under steady state conditions , the serum ferritin level correlates with total body iron stores; thus the serum ferritin FR5R1 is the most convenient laboratory test to estimate iron stores. The ferritin levels measured usually have a direct correlation with the total amount of iron stored in the body. However, ferritin levels may be artificially high in cases of anemia of chronic disease where ferritin is elevated in its capacity as an acute phase protein and not as a marker for iron over load.

A normal ferritin blood level, referred to as the reference interval is determined by many testing laboratories. The ranges can vary between laboratories but are usually between 30-300ng/ml(µg/l) for male, and 6-115ng/ml(µg/l) for females. If the ferritin level is low there is a risk for lack of iron, which could lead to anaemia (Frank and Bryan,1997).

What to make of low ferritin, with normal iron level (Majid shojania,2010)

- In the sequence of event leading to iron deficiency anaemia, the fall of serum ferritin is the earliest manifestation of iron depletion, the fall of serum iron and iron saturation appear next and then anemia develops.
- In partially- treated iron deficiency anemia, serum ferritin remains low when hemoglobin and serum iron have returned to normal. Ferritin returns to normal when iron stores get repleted with futher iron therapy.
- Besides iron deficiency, there are two other conditions that are associated with low serum ferritin. One is hypothyroidism the other is scurvy.
- Variation in daily serum iron is high. Thereôs a significant diurnal variation in serum iron, levels being the highest in the morning and lowest in the evening.
- Serum iron goes up following ingestion of iron containing food but serum ferritin does not.

2.10 TOTAL IRON BINDING CAPACITY (TIBC)

Total iron binding capacity is a medical laboratory test that measures the bloodôs capacity to bind iron with transferrin. It is performed by measuring the maximum amount of iron that it can carry, which indirectly measures transferrin. Since transferrin is the most dynamic carrier. Iron binding capacity is usually increased in iron deficiency anaemia and decreased in hemochromatosis, malignancies, rheumatic fever (Henry,1984). A specimen should be analysed for both iron and iron iron binding capacity because of the need for both values in the differential diagnosis of various types of anaemia and liver diseases.

Interpretation

TIBC is usually performed when anemia, iron deficiency or iron deficiency anemia is suspected.

Condition	**Serum Iron(Highly Variable)**	**TIBC**	**% Transferin saturation**
Iron deficiency anemia	Low	High	Low, as there is in-sufficient iron
Anemia of chronic disease	Low, as the body holds iron intracellularly with ferritin	Low, The body produces less Transferrin to keep iron away from pathogens	Normal
pregnancy or use of hormonal contraception but without iron deficiency.	Normal	High. The liver increases the production of transferrin thus raising TIBC.	Low, as there is excess transferrin with normal serum iron levels

CHAPTER THREE

3.1 MATERIAL AND METHOD

Source of Sample

Two hundred male blood donors were recruited from National blood transfusion centre Enugu, University of Nigeria Teaching Hospital, Ituku Ozalla and two private blood banks in Enugu Urban. Forty apparently healthy non donors were used as controls. All the donors were apparently healthy and fulfilled the viability tests for blood donation. Before starting ethical approval was obtained from the ethics committee of the University Of Nigeria Teaching Hospital, Ituku Ozalla. Informed consent was taken from the blood donors and control group for their willingness to participate in the study. The researcher developed questions to get the information regarding age, sex, dietary habit, past and present illness, infection,surgery/trauma,use of cigarette, frequency of donations , type of donor, last donation interval, number of donations in the preceeding 3years. This was made possible with the help of laboratory assistants working in the blood banks. Subjects receiving iron therapy or had received blood transfusion in the last 12months were excluded. Donors whose haemoglobin concentrations were below 12.5g/dl were excluded. Vegetarians were excluded.

Prior to donation of 450ml of whole blood, 5ml of venous blood sample were drawn aseptically from each subject via the antecubital vein following the standard procedure for veinous blood collection. Two mls of the blood sample were dispensed into tripotassium ethylene diamine tetra acetic acid (k3EDTA) anticoagulant bottles and mixed gently by inversion, for estimation of hemoglobin, packed cell volume(PCV), total white cell count (TWBC) and differential count,

mean cell hemoglobin concentration (MCHC) and blood film. The haematological samples were analysed within one hour of collection. Five ml of samples were put into a plastic plain tube for measurement of serum ferritin levels, serum iron, total iron binding capacity and transferrin saturation. The samples were allowed to clot and the serum was separated into another plain tube and stored at -31^{o}c until used.

The donors were divided into 6 groups according to number of previous donations within the preceeding 3years. The age range was between 19 ï 50years.

Group I ï Non ï donors (controls)

Group II ï Donated once within 3years

Group III ï Donated 2 ï 5 times within 3 years

Group IV - Donated 6 ï 9 times within 3years

Group V ï Donated 10 ï 12 times within 3years

Group VI ï Donated more than 12 times within 3years

Group V and VI donors were considered regular donors. All the regular donors in Group VI were remunerated..

EXCLUSION CRITERIA

- Use of iron supplement
- Anaemia (tested by haemocue method)
- A major trauma / surgery in the last 3 months
- Use of cigarette.
- Blood transfusion within the last 12 months

3.2 METHODS

FERRITIN

3.3 ENZYME IMMUNOASSAY FOR THE QUANTITATIVE DETERMINATION OF HUMAN FERRITIN CONCENTRATION IN HUMAN SERUM. (DIAGNOSTIC AUTOMATION, INC; Cat No: 1601- 18)

Serum ferritin was analyzed using enzyme linked immunosorbent assay method kit (ELISA) purchased from Diagnostic Automation,INC (Cat No: 1601- 18) from Califonia, USA and read with Syntron MSR- 100 micro well reader.

Principle: The ferritin quantitative test is based on a solid phase enzyme linked immuno sorbent assay (ELISA). The assay system utilizes one rabbit anti ferritin antibody for solid phase (microtiter wells) immobilization and a mouse mono clonal anti-ferritin antibody in the antibody enzyme (horse radish peroxidase) conjugate solution. The test sample is allowed to react simultaneously with the antibodies, resulting in the ferritin molecules being sandwiched between the solid phase and enzyme-linked antibodies. After a 45minute incubation at room temperature, the wells are washed with water to remove unbound-labelled antibodies. A solution of TMB reagent is added and incubated at room temperature for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of stop solution and the color is changed to yellow and measured spectrophotometrically at 450 nm. The concentration of ferritin is directly proportional to the color intensity of the test sample.

Assay procedure

1. The desired number of coated wells was secured in the holder.

2. 20µl of standard, specimens and controls was dispensed into appropriate wells
3. 100 ml of Enzyme conjugate reagent was dispensed into each well
4. Mixed for 30 seconds. It is very important to have a complete mixing in the set up.
5. Incubated at room temperature for 45 mins
6. After which the incubation mixture was removed by flicking plate contents into sink.
7. Microtiter wells was rinsed and flicked 5 times with distilled or deionized water
8. The wells was striked sharply into absorbent paper or paper towels to remove all residual water droplets
9. 100 µl of TMB reagent was dispensed into each well and mixed for 10 seconds.
10. Incubated at room temperature in the dark for 20 mins
11. 100 µl of stop solution was added into each well to stop reaction.
12. Mixed for 30 seconds and read at 450 nm with a microtiter plate reader within 15minutes.

LIMITATION OF THE PROCEDURE (Ed.Tietz, 1995)

1. Reliable and reproducible result will be obtained when the assay procedures is carried out with a complete understanding of the package instruction and by good laboratory practice.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

3. Serum samples demonstrating gross lipemia, gross hemolysis or turbidity should not be used with this test
4. The result obtained from the use of this kit should be used only as an adjunct to other diagnostic procedures

Normal ranges: Male -20-250ng/ml

Female -10-120ng/ml

SERUM IRON

3.4 TECO DIAGNOSTICS FOR THE QUANTITATIVE DETERMINATION IRON, TOTAL IRON BINDING CAPACITY IN HUMAN SERUM (TECO DIAGNOSTIC, CALIFORNIA, USA)

The serum iron and total iron binding capacity (TIBC) was analysed and measured spectrophotometrically using Teco sp 203 spectrophotometer(Califonia USA). Transferrin saturation was calculated from the serum iron concentration and TIBC, thus, transferrin saturation= serum iron / TIBC X 100. Test procedure was conducted as described in manufacturers standard operating manual included in the kit.

Principle: The iron in serum is dissociated from its Fe iii transferrin complex by the addition of an acidic buffer containing hydroxylamine. This addition reduces the Fe (iii) to Fe (ii). The chromogenic agent, ferene, forms a highly colored Fe (ii) complex that is measured photometric cally at 560nm.

The unsaturated iron binding capacity (UIBC) is determined by adding Fe (ii) ions to serum so that they bind to the unsaturated iron binding sites on transferrin. The excess Fe (ii) ions are reacted with ferrozine to form the color complex , which is

measured photometrically. The difference between the amount of Fe (ii) added and the amount measured represents the UIBC. The total iron binding capacity(TIBC) is determined by adding the serum iron value to the UIBC value.

PROCEDURE

Serum Iron:

1. Test tubes/cuvettes were labeled; ñBlank ñ,standardò, ñcontrol,ò samplesò etc
2. 2.5 ml iron Buffer was added to all tubes
3. 0.5ml (500 µl) sample was added to respective tubes and mixed. Note: 500µl iron free water was added to blank.
4. Zero spectrophotometer at 560 nm
5. Absorbance of all tubes was read and recorded (A1 reading)
6. 0.05µl (50 µl) of iron color regent was added to all tubes and mixed
7. All tubes were placed in the heating bath at 37ºc for 10 mins
8. Zero instrument at 560nm with reagent blank
9. Absorbance of all the tubes was read and recorded (A_2 reading)

CALCULATION : $\frac{A_2 \text{ test} - A_1 \text{ test} \times \text{conc. of std}}{A_2 \text{ std} - A_1 \text{ std}} = \text{total iron } \mu g/dl$

UIBC

1. Test tubes/cuvettes were labelled, blank, standard, control, test
2. 2.0ml UIBC buffer reagent was added to all tubes
3. 1.0ml iron free water was added to blank and mixed

4. 0.5 ml (500µl) iron free water plus 0.5ml(500µl) standard was addaed to standard and mixed
5. 0.5ml (500µl) respective samples plus 0.5ml(500µl) iron standard was added to tes and mixed
6. Zero spectrophotometer at 560nm with reagent blank
7. Absorbance of all tubes was read and recorded (A_1reading)
8. 0.05ml (50ul) of iron color reagent was added to all tubes and mixed
9. All the tubes was placed in a heating bath at 37^0C for ten minutes
10. Zero spectrophotometer at 560nm with reagent blank
11. Absorbance of all the tubes was read and recorded (A_2 reading)

UIBC CALCULATION

$$\text{Conc. Of std-} \left[\frac{A_2\text{test}-A_1\text{test}}{A_2\text{std}-A_2\text{std}} \times \text{conc. of std}\right] = \text{UIBC µg/dl}$$

3.5 HEMOGLOBIN ESTIMATION

Haemocue method

Principle:the reaction in the cuvette is a modified axidemethemoglobin reaction. The erythrocyte membranes are disintegrated by sodium deoxycholate, releasing the hemoglobin. Sodium nitrite converts the hemoglobin iron from the ferrous to the ferric state to form methemoglobin, which then combines with azide to form azide methemoglobin. The photometer uses a double wavelength measuring method, 570nm and 880nm, for compensation of turbidity.

Sample materials: capillary, venous or arterial whole blood.

Procedure

- The forefinger or thumb was cleansed and lanced
- The finger was lightly pressed and the first two drops of blood wiped off before collecting into the cuvette.
- The cuvett was filled directly with the blood. If the cuvette is not completely filled, a new curvette is used to start over again
- Excess blood from the outside of the curvette was wiped off and presence of air bubbles eliminated.
- The filled curette was placed into cuvette holder and gently slid into measuring position.
- The result appears and is recorded.
- The instrument is cleansed with alcohol prep after each use

Normal ranges men- 13.5- 16.0g/dl

Normal ranges women- 11.5-13.5g/dl

3.6 PACKED CELL VOLUME BY MICRO HAEMATOCRIT METHOD (JELKMANN W 2004)

Principle: the packed cell volume (PCV) can be determined by centrifuging heparinized blood in a capillary tube at 10,000rpm for 5minutes. This separates the blood into layers. The volume of packed red blood cells divided by the total volume of blood sample gives the Pcv (Jelkmann, 2004)

Materials: capillary tubes, plain or heparinized, burner or clay sealants, micro hematocrit centrifuge, microhematocrit reader.

PROCEDURE

- The capillary tube was filled two ï thirds to three quarters fill with well mixed, oxalated venous blood
- One end of the tube was sealed with flame and placed in micro heamatocrit centrifuge
- The centrifuge was spurn at 10,000 to12,000 rpm for 5mins
- The tube was then placed in the microheamatocrit reader and the result recorded in percentage.

Normal Range: Men- 42-52%

Women- 37-47%

3.7 TOTAL WHITE BLOOD CELL COUNT (FRANKHAUSER, 2001)

The total white cell count determines the number of white cell per cubic millimeter of blood.

Principle: Turks solution which is composed of gential violet and 6% glacial acetic acid destroys red blood cells within a blood sample and stains the nuclei of the white blood cell, making them easier to be seen and counted.

PROCEDURE

1. 2 drops of anticoagulated blood was placed in a clean tube
2. 2 drops of turks solution was added into the same tube and mixed. A cover slip was placed over the neubeaur counting chamber.
3. The mixture was allowed to flow into the chamber by using the tip of a micropipette. Over filling was avoided

4. This was allowed to settle for 1 minute.
5. It was then examined under the microscope using x 10 objective. Only cells within the indicated squares were counted. Cells touching the right and left lines were ignored. The equipment was cleaned up after each use. Wbcs were calculated in cumm

Normal Range- 4,800-10,800cumm

3.8 DIFFERENTIAL WHITE BLOOD CELL COUNT USING LEISHMAN STAIN (MONICA CHEESBROUGH)

Principle: Leishman stain contains methylene blue dye, a basic dye, which gives colour to an acidic component and Eosin dye, an acidic dye, which gives color to a basic component. These dye differentiate the different component of blood.

Procedure

- Thin films of blood were prepared from EDTA anticoagulated blood sample
- The films were labelled with numbering and date and allowed to dry
- The leishman stain was flooded on the slides and allowed for 2mins. After which it was diluted with water and allowed for further 10mins.
- The stain was then washed off the slides with flowing water. The back of the slides were cleaned and then allowed to dry. The slides were viewed under a microscope using x 100 objective with oil immersion. The percentage of different types of wbc were counted per-ten fields and recorded.

Normal ranges - Neutrophilst: 40-60%

Lymphocyte: 29-40%

Monocytes: 2-8%

Eosinophils: 1-4%

Basophils: 0.5-1%

Band: 0-3%

STATISTICS

Statistical tools used were Mean ±2SD, one way analysis of variance (ANOVA) and Dunns multiple comparison test. An alpha value of <0.05 denoted a statistically significant difference .Statistical package used was graph Pad Prism version 5.03.

CHAPTER FOUR

4.1 RESULT

The mean ± 2SD in ferritin level showed a significant decrease across the groups (P< 0.0001).The ferritin levels in the control group; 56.77± 33.83ng/ml, was higher than that in donors in groups ii, iii, iv, v, & vi; 52.67±40.47ng/ml, 51 .20±23.25ng/ml, 21.46±6.16ng/ml, 16.11±21.4ng/ml, 6.76±5.54ng/ml respectively. The serum ferritin concentrations were statistically significantly different when comparing regular donors in groups iv, v, & vi with group ii and iii donors as shown in Table 1.

The serum iron level decreased significantly (P < 0.0001) across the groups. In group i(controls) the mean serum iron concentration was 94.l7±27.66µg/ml, while in group ii donors it was 113.8±71.2µg/ml. In groups iii, iv, v, & vi, the mean serum iron concentration was 83.71±10.84µg/ml, 72.73±l0.98µg/ml, 70.00±11 .62µg/ml,& 65.86±15.57µg/ml respectively(Fig. 2). The mean serum iron concentration was significantly higher in group i(controls), group ii, & iii donors compared to donors in groups iv, v, & vi, who had given blood six times or more in the preceding 3 years.

The TIBC increased significantly (P< 0.0001) across the groups with mean values 371.2 ± 76.14µg/ml, 455.4 ± 111.9µg/ml and 481.0 ± 98.62µg/ml for groups iv, v, & vi respectively compared to; 309.3 ± 72.0µg/ml, 333.7 ± 86.0µg/ml, 320.2 ± 82.2µg/ml for groups i, ii and iii respectively. (Fig. 3).

The mean ± 2SD for transferrin saturation showed a significant decrease(P <0.0001)across the group(Fig. 4), with the donors in group vi, v, & vi having 20.50

± 5.66%, 23.60 ± 10.74%, 20.64 ± 8.22% compared to groups i (controls), ii, & iii donors;31.38±10.47%, 33.47±15.4%, 34.47±12.8% respectively.

The mean ± 2SD for Hb and Pcv of the subjects were significantly reduced ($p < 0.0001$) with donors in goups v and vi having 13.64± 0.76g/dl; 40.40± 3.27% and 13.78± 0.63g/dl; 39.79±4.72% respectively when compared with group i(control), group ii and iii; 14.77±0.87g/dl; 44.01±3.12%; 15.12±1.14g/dl; 44.53±3.83%; 15.20±1.24g/dl; 44.73±3.80% respectively (Fig 5 and 6). There was no significant difference in Hb and PCV of donors in group iv when compared with those in groups i(controls), ii, v and vi. Also there was no significant difference in the MCHC and WBC total across the groups (Fig 7and 8) respectively.

There was a significant difference when comparing groups iii with group iv and group iv with group vi, with ferritin showing significant difference for these groups while the serum iron did not differ significantly in the groups. Also there was no significant difference in the iron and haematological parameters of subjects in group i(controls) compared to the donors in groups ii and iii in 3years.

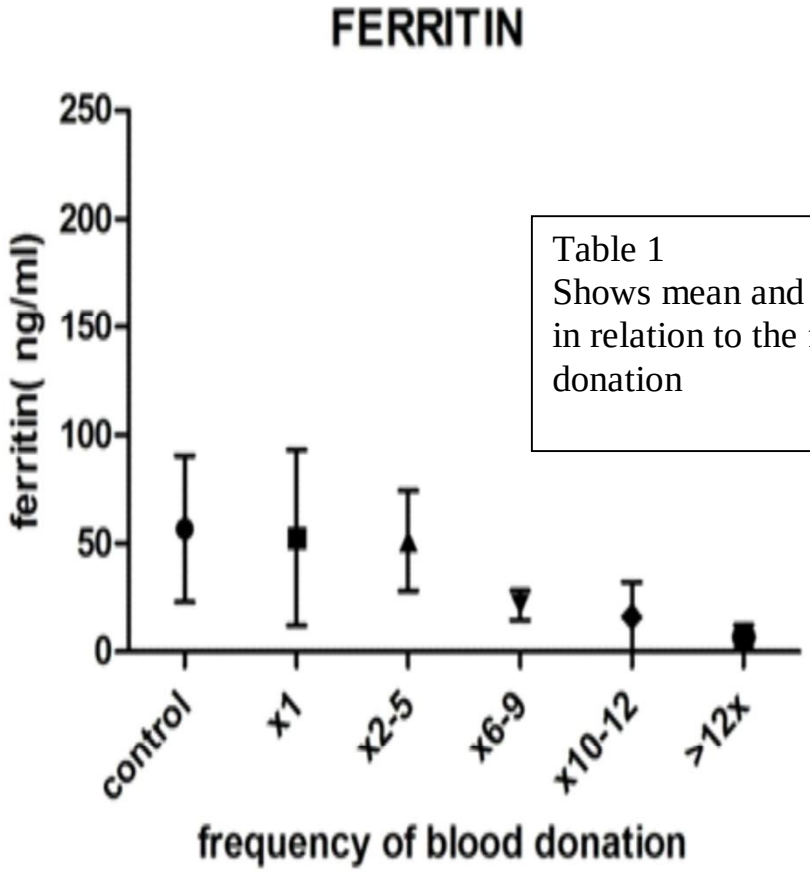


Table 1
Shows mean and SD of Ferritin in relation to the frequency of donation

	control	x1	x2-5	x6-9	x10-12	>12x
Mean	56.77	52.67	51.20	21.46	16.11	6.763
Std. Deviation	33.83	40.47	23.25	6.765	16.06	5.544

P value	< 0.0001

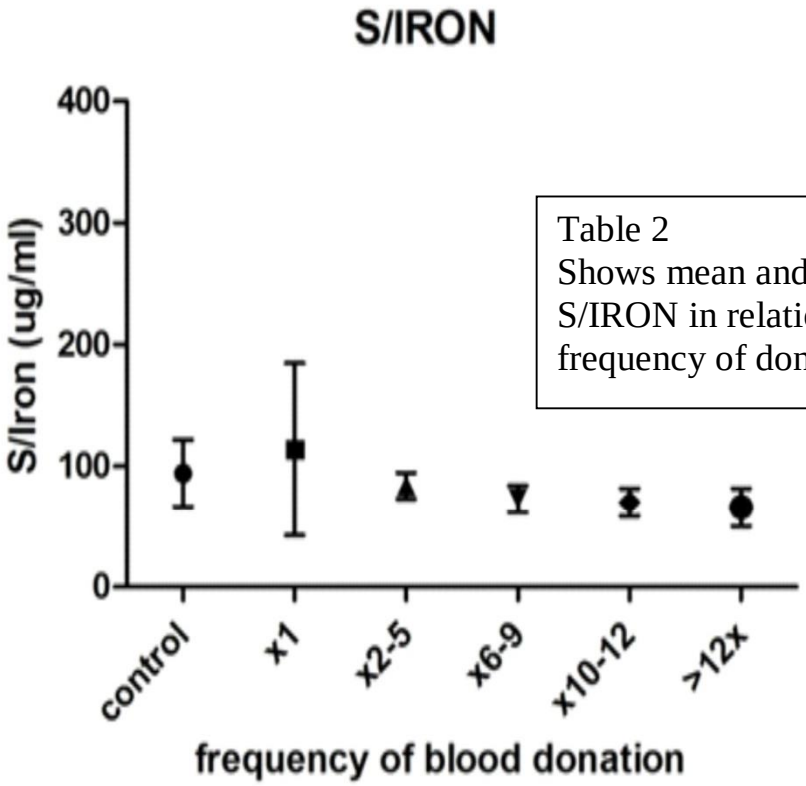
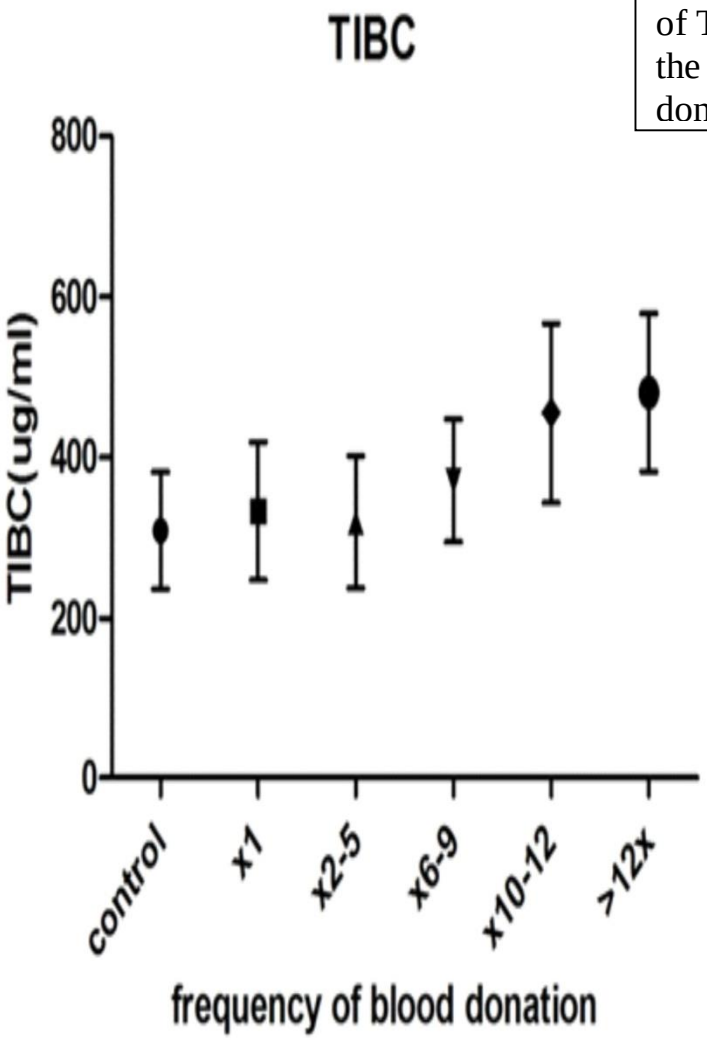


Table 2
Shows mean and SD of S/IRON in relation to the frequency of donation

	control	x1	x2-5	x6-9	x10-12	>12x
Mean	94.17	113.8	83.71	72.73	70.00	65.86
Std. Deviation	27.66	71.20	10.84	10.98	11.62	15.57

P value	< 0.0001

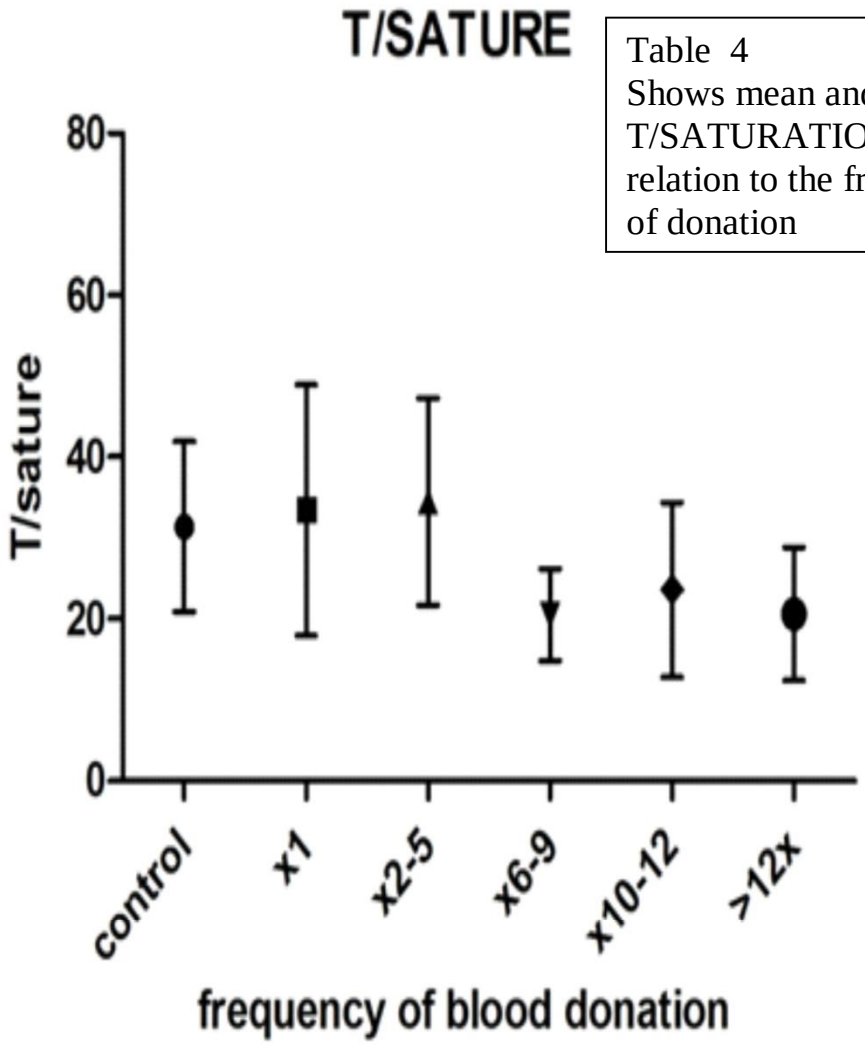
Table 3
Shows mean and SD of TIBC in relation to the frequency of donation



	control	x1	x2-5	x6-9	x10-12	>12x
Mean	309.3	333.7	320.2	371.2	455.4	481.0
Std. Deviation	72.70	86.09	82.28	76.14	111.9	98.62

P value	< 0.0001

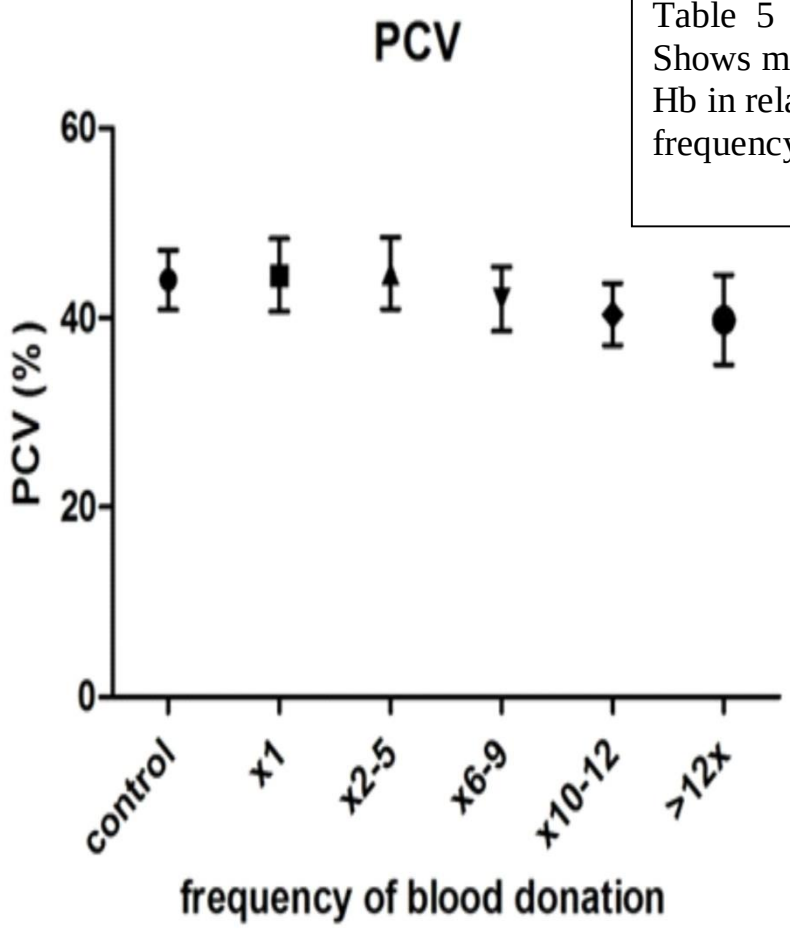
Table 4
Shows mean and SD of T/SATURATION in relation to the frequency of donation



	control	x1	x2-5	x6-9	x10-12	>12x
Mean	31.38	33.47	34.47	20.50	23.60	20.64
Std. Deviation	10.47	15.48	12.80	5.666	10.74	8.223

P value	< 0.0001

Table 5
Shows mean and SD of Hb in relation to the frequency of donation



	control	x1	x2-5	x6-9	x10-12	>12x
Mean	44.01	44.53	44.73	42.02	40.40	39.79
Std. Deviation	3.122	3.832	3.800	3.373	3.275	4.725
P value	< 0.0001					

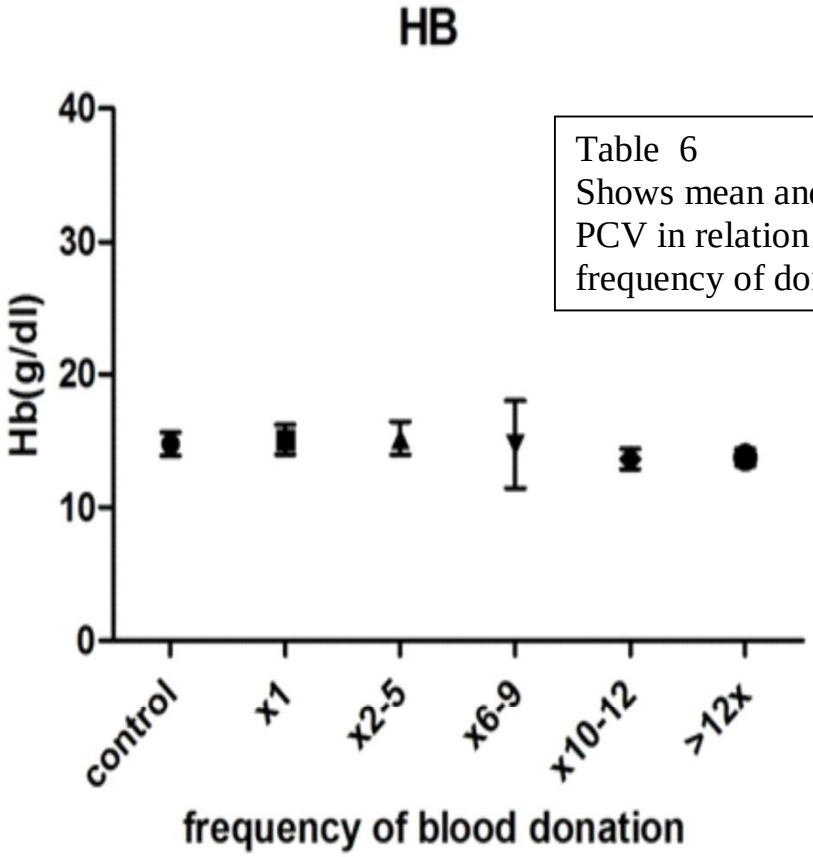
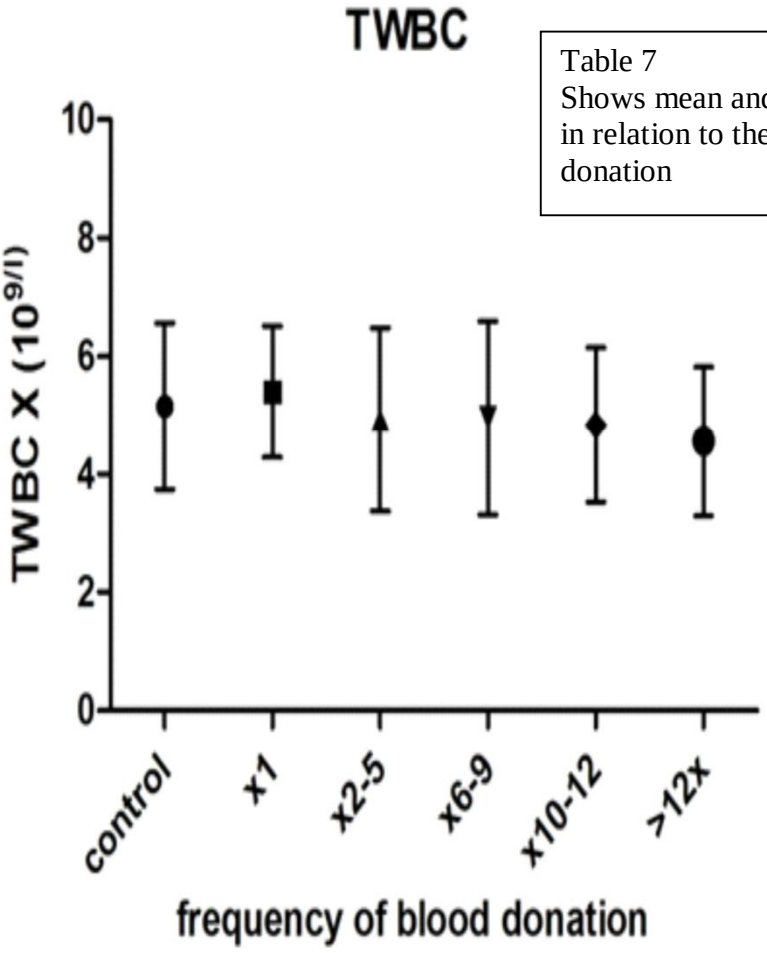


Table 6
Shows mean and SD of PCV in relation to the frequency of donation

	control	x1	x2-5	x6-9	x10-12	>12x
Mean	14.77	15.12	15.20	14.73	13.64	13.78
Std. Deviation	0.8736	1.143	1.243	3.285	0.7649	0.6396

P value	< 0.0001

Table 7
Shows mean and SD of MCHC in relation to the frequency of donation



	control	x1	x2-5	x6-9	x10-12	>12x
Mean	5.153	5.398	4.929	4.955	4.838	4.560
Std. Deviation	1.411	1.116	1.556	1.641	1.306	1.262

P value	0.0784

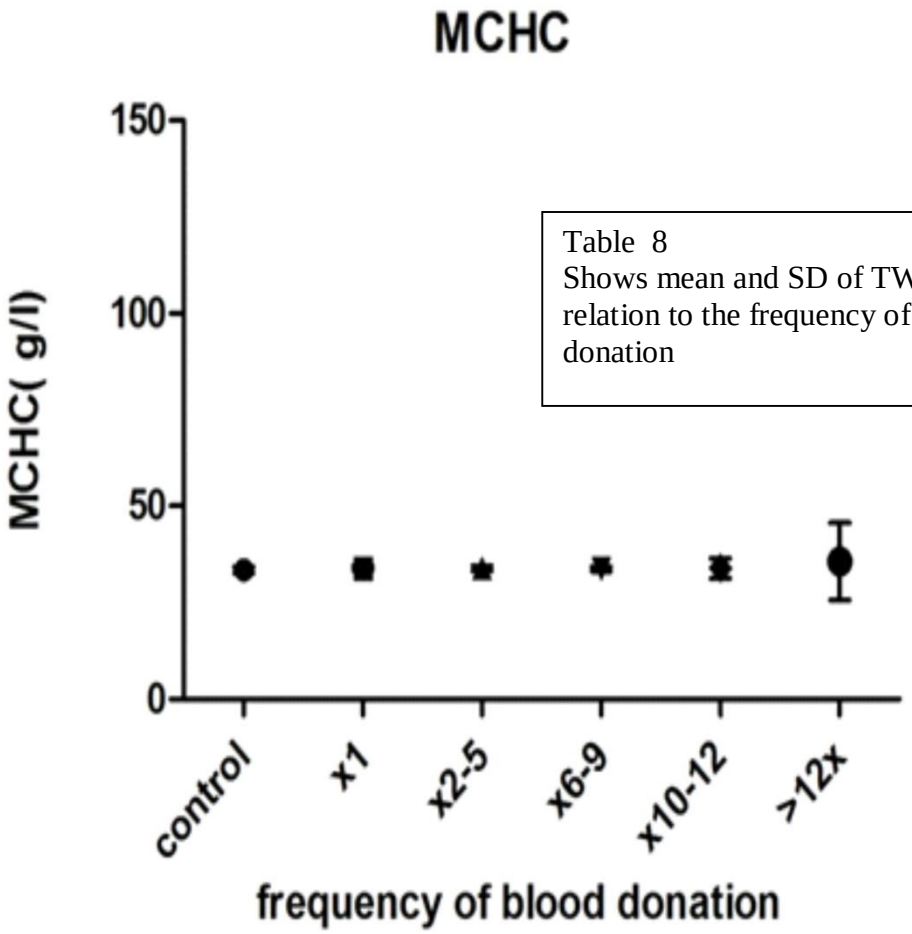


Table 8
Shows mean and SD of TWBC in relation to the frequency of donation

	control	x1	x2-5	x6-9	x10-12	>12x
Mean	33.45	33.91	33.95	33.72	33.91	35.66
Std. Deviation	0.7507	0.4730	0.6302	0.5553	2.537	9.999

P value	0.0024

CHAPTER FIVE

5.1 DISCUSSION

Iron is a vital element in human metabolism. It plays a central role in erythropoiesis and is also involved in many other processes in all the tissues of the body (Brittenham, 1991). The potential for an individual donor to give blood without developing iron deficiency anaemia is dependent on many factors such as differences in nutritional iron intake, the prevalence of iron deficiency in the particular population, and the frequency of blood donation (Milman *et al*, 1984).

Recent studies have shown that the frequency of iron deficiency is high in blood donors and more dependent on the frequency of donation than on the accumulated number of donations (Simon *et al*, 1981).

One of the most frequent observation in long term blood donors is chronic iron deficiency . Iron depletion is the earliest stage of iron deficiency and signifies that iron stores are decreased or absent, but the serum iron concentration and blood haemoglobin levels are normal. Non anemic iron deficiency is a some what more advanced stage of iron deficiency, characterized by decreased or absent iron storage usually a low serum iron concentration and low blood haemoglobin con centration, but without anaemia. Blood banks have the responsibility to protect blood donors which includes preventing anaemia among them. In tropical Africa , for example Nigeria, non-remunerated donors are in very short supply, sometimes because of certain superstitious beliefs relating to blood, resulting in commercial donation and this makes the donors not to reveal all their medical history, life styles or frequency of donations (Ukaejiofo,2009). In this study, it was observed that a donation frequency of more than 2 units per year could not be compensated

by iron absorption and resulted in reduction in iron storage. Early detection of iron deficiency among blood donors would allow appropriate readjustment of donation interval and would guide the use of iron supplementation to reduce the rate of donor deferral for low haemoglobin.

This study confirmed the findings of previous studies, in that iron depletion increased with frequency of blood donations and consequently erythropoiesis with iron deficiency which was evident in subjects who donated 10-12 times and >12 times in 3years. The values of iron parameters (ferritin, TIBC, serum iron ,transferrin saturation) and some haematological parameters(Hb, and pcv) were significantly decreased or increased in the regular donors who donated 10-12 times or >12 times in 3years compared to other groups of donors. This could be attributed to the frequency of donations by this group of donors who donated blood for more than four times a year. Similarly, there was a statistically significant decrease in iron parameters of subjects who donated 6-9 times in 3years which could be due to the non-intake of haematinics or poor diet, whom inspite of their acceptable standard of frequency of donations per annum had a significant difference when compared with controls and other group of donors with the exception of 2-5 times donors and >12 times donors whose serum ferritin was affected but not serum iron.

Similarly, there was a statistically significant increase in haemoglobin concentration and packed cell volume ($p<0.05$) in the control group when compared with those who donated 10-12 times and >12 times in 3 years. This is likely to be due to the frequency of donations leading to iron deficient erythropoiesis. The Wbc total and differencial of both the donors and control group shows no significant difference. This may be due to the fact that both the controls

and donors were apparently healthy and had no symptoms of any chronic infection. The MCHC did not differ significantly ($p > 0.05$) in the donors when compared with the control group. The most likely explanation might be due to the slight decrease though statistically significant in the Hb concentration and Pcv mean values. In contrast, Nubilla *et al,* 2014, observed that there were statistically significant increase in the red blood cell count, hemoglobin concentration, packed cell volume,total white blood cell and eosinophils counts in donors when compared with the control group. In their study, they thought that the donors prepared themselves better for donation by taking haematinics and improved diet, though in that study donors where not grouped according to number of donation. In line with this study, Simon *et al* (1981), showed that 8% of men and 38% of women have reduced iron stores, as assessed by serum ferritin status, after five donations. In a large Danish study, the prevalence of depleted iron stores was found to be higher in donors than in people who did not donate blood.

In Nigeria, Usanga (1990), observed that the mean ferritin concentration of 64.75ng/ml ± 4.6 in non donors was significantly higher than the mean value of 49.19ng/ml among blood donors suggesting that some blood donors may have prelatent or latent iron deficiency at the time of donation and become iron deficient after blood donation. In a study in portharcourt, Nigeria by Zacheaus and Baribefe, (2008) , it was found out that in the three categories of donors (relative,voluntary and regular donors),the regular donors were found to be most adversely affected as shown by the reduction in mean values of both haematological and biochemical iron parameters. In contrast, Akpotuzor *et al* (2008), concluded that there was no observable difference in biochemical iron parameters between regular donors and healthy controls. Similarly, Adewumi *et al*(2013), in their findings suggested that

haemoglobin concentration, packed cell volume and serum iron levels are not significantly affected by regular blood donations.

Oluboyede et al 1983, evaluated serum ferritin levels and haematological parameters in a Nigerian population and observed that haematological values fell within the reported range for healthy population in other parts of the world but the mean serum ferritin value in the male population was 72.4ng/ml compared with the female value of 34.2ng/ml.

In line with this study, Okpakan *et al,* (2012), showed that the PCV, Hb, serum ferritin, TIBC, transferrin saturation and serum iron of 2^{nd}, 3^{rd}, 4^{th}, and 5^{th} time blood donors were significantly reduced ($p < 0.05$) progressively as frequency of donation increased when compared with the 1^{st} time blood donors. The present study has shown that Hb , Pcv, MCHC, Serum iron, TIBC, Transferrin saturation and serum ferritin of once and 2 - 5 times donations in 3 years in this locality did not differ significantly from that of controls suggesting that it may be more appropriate for donors in this locality to donate twice in a year rather than three or four times as had been the case . This findings is in line with that of Okpakan *et al*, 2012 in Calabar, Nigeria. On the other hand, the subjects who donated blood for 10-12 times or >12 times in 3 years were mostly affected. The reason may be attributed to the poor economic status of most of these regular donors as they donate for monetary benefits. Also poor nutritional status and lack of awareness, are also contributory factors to the low iron and haematological parameters observed especially in the subjects who donated 10 ï 12 times and > 12 times in 3years.

5.2 CONCLUSION

Iron depletion is common among regular blood donors in Enugu state, Nigeria, despite these donors being eligible to give blood according to the current guidelines (Hb>12.5) . This study observed that Haemoglobin, Packed cell volume, Serum iron, Serum ferritin, Transferrin saturation, TIBC, were significantly affected in the regular blood donors especially those who have had more than six donations in 3 years when compared with apparently healthy controls.

5.3 RECOMMENDATIONS

- ⇨ There is need to educate regular voluntary blood donors about iron deficiency because this can lead to iron deficiency anaemia if precautions are not taken.
- ⇨ The national blood Transfusion services should effectively regulate blood donation system by creating awareness and making sure that donors adhere strictly to frequency of blood donation pertaining to their locality because iron depletion may be more prevalent in some localities than others.
- ⇨ It may be necessary to include serum ferritin as one of the parameters to be assayed on blood donors and consequently, improve the well being of donors.
- ⇨ Remunerated blood donations should be discouraged while voluntary donation should be encouraged because remuneration may cause the donors not to reveal all their medical history, lifestyle and frequency of blood donations.

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APPENDIX 1

Materials for Quantitative Determination of Iron and TIBC in Serum

1. Iron Buffer reagent
2. UIBC Buffer reagent
3. Iron color reagent
4. Iron standard (500µg/dl)

Materials for Quantitative Determination of Ferritin in Serum

- Anitibody coated microtiter plate with 96 wells
- Enzyme conjugate reagent, 13 ml
- Ferritin reference standards, containing 0,15,80,250,500 and 1000ng/ml, liquid, 0.5ml ready to use
- TMB reagent (one step), 11 ml
- Stop solution (1N HCL), 11 ml