

THE IRON STATUS OF ZIMBABWE BLOOD DONORS

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Abstract

Background

Transfusion medicine is a distinct and expanding discipline with a vital role in the health care system, according to the permanent secretary in the ministry of health and child welfare. In Zimbabwe the number of people who failed the copper sulphate test (haemoglobin screening test) shot up from 1249 in 2011 to 2360 in 2012 (89% increase). Failing this screening test is caused among other things by low haemoglobin levels which in turn are caused by low iron levels in the body. Checking haemoglobin levels alone is not enough to check the status of the donors, but a complete iron profile is necessary to ascertain the actual status of the blood donors.

Aim

To find out the iron status of blood donors in Zimbabwe.

Materials and Methods

A cross sectional study was done on 190 blood donors at the National Blood Service Zimbabwe (NBSZ). Left over samples were analysed for serum iron, serum ferritin, TIBC (total iron binding capacity) and transferrin saturation at Premier Clinical Laboratory. Donors recruited into the study ranged from first time donors to donors who had donated up to 8 times in the past two years.

Results

A total of 190 (109 males and 81 females) blood donors participated in this study. The median age was 23 years and the inter quartile range was 19.0-27 years. The range of the age was from 16 years to 67 years. The median number of donations was 4 and the interquartile

range was 5-6 units over the two year period under study. The median serum iron levels was 13 $\mu\text{mol/L}$ with the inter quartile ranges from 10.0-17.8 $\mu\text{mol/L}$. For ferritin the median value was 30 ng/ml with the inter quartile ranges from 18.0-56.8 ng/ml. The median for TIBC was 74.1 $\mu\text{mol/L}$ with the inter quartile ranges from 63.3-82.3 $\mu\text{mol/L}$. For transferrin saturation the median was 17.6 $\mu\text{mol/L}$ with the inter quartile ranges from 12.0-26.9 %. The statistical analysis was done by the ANOVA test. As for serum ferritin levels there was statically significant differences in the mean ferritin levels between group 1 and groups 6,7 and 8 ($p<0.01$) for men. For females there was statistically significant difference in the mean ferritin levels between the control group and group 6 and 7 ($p <0.05$). The overall prevalence of iron deficiency in the study population was 13.2% and the prevalence of reduced iron stores was 37.4 %.

Conclusion

These findings suggest that repeated blood donation causes a reduction in the iron stores of the blood donors in Zimbabwe and there is need to include biochemical markers, (serum iron, serum ferritin, TIBC and transferrin saturation) in the screening of blood donors, especially from the fifth unit.

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List of Abbreviations

CD	-	Cluster of Differentiation
CDC	-	Center for Disease Control
CRP	-	C-reactive protein
CV	-	Coefficient of Variation
HIV	-	Human Immunodeficiency Virus
HLA	-	Human Leucocyte Antigen
JREC	-	Joint Research Ethics Committee
LIS	-	Laboratory Information System
MCH	-	Mean Corpuscular Haemoglobin
MCHC	-	Mean Corpuscular Haemoglobin Concentration
NBSZ	-	National Blood Service Zimbabwe
NTBI	-	Non-transferrin bound Iron
PCV	-	Packed Cell Volume
Th	-	T helper lymphocytes
TIBC	-	Total Iron Binding Capacity
TSAT	-	Transferrin Saturation
TTI	-	Transfusion Transmissible Infections
WHO	-	World Health Organisation

Chapter 1

1.0 INTRODUCTION

1.1 BACKGROUND AND LITERATURE REVIEW

Transfusion medicine is a distinct and expanding discipline with a vital role in the health care system, according to the permanent secretary in the ministry of health and child welfare¹. The discipline is concerned with many subjects that include: public education, donor recruitment and retention, donor selection, counselling and blood collection, laboratory processing, organisation and management of the blood service, and working with the clinicians to promote the appropriate clinical use of blood and blood products¹. Providing safe and adequate blood should be an integral part of every country's national health care policy and infrastructure². Blood donation rate in high-income countries is 39.2 donations per 1000 population and 4.0 donations in low income countries².

The National Blood Service Zimbabwe (NBSZ) in 2012 alone collected 81 779 units of blood. Seventy per cent of the annual blood collections come from scholars. These scholars are urged to continue donating blood after leaving school and they will join a club called Pledge 25 club. Pledge 25 club is a special club of young and healthy citizens who pledge to regularly donate safe units of blood at least 25 times in their lives. The primary aim of forming this club was to allow for a smooth transition from school donors into safe adult donors thereby effortlessly creating a future pool of informed adult blood donors, bearing in mind that the recruitment of a new donor is more expensive than the retention of a donor³.

An adequate and reliable supply of safe blood can be assured by a stable base of regular, voluntary donors³. Many blood transfusion services wait until they lose a donor for health

reasons to take remedial action but it will be better if the health of a donor is monitored during the course of the donation period, before that donor is lost. Donors are lost through the process of deferral which could be for haematological and serological reasons. Prior to donation donors go through a screening process which involves physical examination with medical personnel to check that they are in good health. All blood donors are asked questions about their medical history to help to determine whether they can safely donate blood without experiencing any negative health effects. These include questions to rule out heart and lung diseases, seizures, recent surgery, pregnancy, possible exposure to parasitic diseases, bacterial infection and cancer⁴. Loss of units from both first time and repeat donors due to temporary deferral are more common events than losses due to disease marker testing. Some of these losses may be avoidable and could increase the blood supply without having to recruit new donors⁵.

In several studies done around the world low haemoglobin was the major reason for deferral^{5, 6, 7, 8}. In Zimbabwe the number of people who failed the copper sulphate test (haemoglobin screening test) shot up from 1249 in 2011 to 2360 in 2012 (89% increase). This is consistent with findings by the World Health Organisation (WHO) who pointed out that anaemia is one of the most common and intractable nutritional problems in the world today. Two billion people are estimated to be anaemic. The main causes of anaemia are: dietary iron deficiency, infectious diseases such as malaria, hookworm infections and schistosomiasis, deficiency of vitamin B12 and vitamin A and inherited conditions that affect red blood cells⁹.

Different studies done in different countries have different findings on the iron status in blood donors. Adediran et al (2013) found out that the haemoglobin concentration, packed cell volume (PCV), and serum iron levels were not significantly affected by regular blood donation and that regular blood donors appear to have reduced iron stores compared with

controls¹⁰. Flesland et al also found out that there was no significant difference in the haemoglobin concentration between first time and regular donors¹¹. No significant difference was noted by Vilzu et al in the ferritin levels between controls and donors donating less than 20 units¹². However a majority of studies ^{13, 14, 15} have shown that blood donation has significant influence on iron stores of blood donors. An inverse relationship of serum iron, ferritin and transferrin saturation with the number of blood donations was obtained in a study by Mahida¹⁶. A study done at Casablanca blood transfusion centre in Morocco found out that the haemoglobin concentration, serum ferritin, serum iron were significantly lower in frequent female blood donors when compared with results of same parameters in first time female blood donors, 43% versus 14% respectively¹⁷. Badar et al (2002) found a statistically significant decrease in the mean serum ferritin level of donors donating more than 3 times in 2 years¹⁸. Brittenham (2011) pointed out that increase in the number of red blood cells donations in the preceding two years was the strongest predictor of iron deficiency among frequent donors¹⁹.

A genetic disorder in which there is excessive accumulation of iron in the body (iron overload) is called hereditary haemochromatosis. It is an autosomal recessive disorder associated with C282Y and H63D mutations in the HFE gene²⁰. The prevalence of iron overload in sub-Saharan Africa region is about 10 % in adults in some rural communities which is the highest in the world²¹. Alexander (2000) pointed out that iron overload could be due to diet and genetic makeup²². African iron overload has been recognised in sub-Saharan Africa for seventy years²³. In 2009 it was also found out that iron overload could also be due to increased dietary iron and a genetic defect not associated with the HLA-locus ²³. African iron overload has clinical consequences. It is a cause of hepatic fibrosis and cirrhosis, and associations with diabetes mellitus, peritonitis, scurvy and osteoporosis have been described.

It can also cause hepatocellular carcinoma. The disorder is associated with a poor outcome in tuberculosis, an infection that is highly prevalent in sub-Saharan Africa²³. It has become imperative for us to study the iron stores in the Zimbabwean donor population.

Donating blood improves the overall cardiovascular health of the donor. Increased levels of iron in the blood raises the chance of heart disease. Regular blood donation reduces the chance of heart attack by 88% and lowers the risk of severe cardiovascular events such as stroke by 33%²⁴. Iron can be a catalyst in the Haber–Weiss reaction which leads to the generation hydroxyl radicals, hydrogen peroxide and superoxide. Having iron available to participate in free radical reactions is important in the pathophysiology of diseases such as diabetes and cardiovascular disorders²⁵. Iron was also found to have negative effects on vascular function by increasing reactive oxygen species locally, decreasing the bioavailability of nitric oxide, impairing vasorelaxation, and promoting platelet adhesion and aggregation²⁶. Iron is also key in the functioning of the immune system. It is essential for erythropoiesis and also it is an active component of several enzymes also relevant for the growth and function of immune cells. Iron is involved in immune effector pathways of neutrophils and macrophages again by formation of toxic hydroxyl radicals²⁷. Iron restriction affects the proliferation and differentiation of B and Th1 lymphocytes, while iron overload leads to dysfunction of natural killer cells, impaired neutrophil cytotoxicity and changes in the ratio of CD4+ to CD8+ lymphocytes²⁷. Imbalances in iron homeostasis influence cytokine activities and cell mediated immune effector mechanisms of macrophages²⁷. High serum values were associated with an increased risk of myocardial infarction in a Canadian study²⁸. Blood donation also enhances production of new red blood cells by the bone marrow thereby refreshing the blood²⁹.

Iron is an essential element that is lost with each blood donation. In order for a donor to compensate for the iron lost in donating blood, iron is mobilised from the body's iron stores and absorption from the diet is increased. However this balance is often difficult to maintain in regular blood donors since there is an on-going blood loss²⁹. Physicians can prescribe therapeutic phlebotomies for patients who have too much iron stored in their bodies and whose haemoglobin levels are sufficient to tolerate blood removal³⁰. A donor on average donates about 450ml of blood per donation. This contains about 225mg of iron. In the absence of iron deficiency the haemoglobin levels return to normal after 3-4 weeks. Therefore adequate iron stores are important in the maintenance of donor health³¹. Chronic iron deficiency is a well-recognised complication of regular blood donation³¹. In Zimbabwe women donate a maximum of 3 units and men donate a maximum of 4 units per year. Regular blood donation increases demand of iron and can lead to depletion of iron stores followed by development of various degrees of iron deficiency³³. Iron deficiency anaemia occurs due to lack of sufficient iron to produce haemoglobin. It is the most common type of anaemia worldwide³⁴. Iron deficiency anaemia can be caused by chronic blood loss, inadequate intake from the diet or absorption and also by pregnancy³⁴. Iron deficiency can delay normal motor function or mental function, increased fatigability, loss of memory, glossitis, difficulty maintaining temperature and in pregnancy it can increase the risk of preterm babies³⁵.

Prior to donation donors are screened by the copper sulphate screening method which is based on the principle of specific gravity. The American Association of blood banks has a standard of minimum haemoglobin of 13.5 g/dl for males and 12.5 g/dl for female donors. The same cut off values have also been adopted by the Ministry Of Health and Child Welfare and as a result these are used by the National Blood Service Zimbabwe (NBSZ)³⁶. Jeremiah

etal (2010) pointed out that haemoglobin cut-off levels at the time of donor screening do not appear to be predictive of iron deficiency as shown by a significant proportion of individuals with low ferritin levels who had a haemoglobin concentration above the cut off³⁷. The use of haemoglobin for screening has been reported to have poor sensitivity in the detection of people with low haemoglobin, in the early stages of iron deficiency³⁸. An accurate diagnosis of a state of iron deficiency requires several laboratory tests. Several studies have also pointed out that iron stores may be depleted in donors with haemoglobin levels above the arbitrary defined limit of anaemia³⁸.

Screening donors' serum ferritin levels at the time of first donation and subsequently once every year is a very rational way to pick up iron deficiency in a voluntary blood donor population. It has been shown that increase in the number of donations results in an increase in the frequency of depleted iron stores and subsequently in erythropoiesis with iron deficiency, although the level of haemoglobin remained acceptable for blood donation³⁸. Szymczyk-Nuzka et al (2003) also found out in another study that there was a high incidence of iron depletion in regular whole blood donors with a normal blood count³⁹. Iron depletion is the earliest stage of iron deficiency and signifies that iron stores are decreased or absent but haemoglobin levels are normal. Only when iron stores are insufficient for haeme synthesis (that is iron deficiency anaemia) do haemoglobin levels and red cell indices begin to decrease⁴⁰. Several blood tests can be used for the evaluation of the body iron stores in blood donors. These include serum iron, total iron binding capacity, serum ferritin, transferrin saturation, soluble transferrin receptor and packed cell volume, haemoglobin and the red cell parameters such as MCHC and MCH. Additional variables that have been investigated include erythrocyte ferritin and zinc protoporphyrin. The iron content of the body is kept constant between the amount absorbed and amount lost and this amount also depends upon the

interaction of foods, drugs and abnormal components of diet. Diagnosis of iron deficiency in most patients can be made based on the measurement of a low serum iron and low serum ferritin with an elevated total iron binding capacity (TIBC) ⁴⁰.

Studies on the diagnosis of iron deficient states have been complicated by the absence of clear cut reference method to detect the biochemical iron deficiency⁴¹. Iron staining of the bone marrow is the gold standard for diagnosis of iron deficiency but the invasive nature of this procedure limits its use⁴¹. A less invasive standard for iron deficiency is based on the haematologic response to iron replacement therapy; a increase of the reticulocyte count or reticulocyte index after oral or intravenous iron replacement therapy reveals the presence of iron deficiency⁴¹. However, the WHO/CDC expert consultation recommended that haemoglobin and ferritin are the most useful indicators for assessment of iron deficiency. They also recommended that transferrin receptor should be added to the two in places where infection is common⁴².

Iron contained in serum (or plasma) is normally bound to the protein transferrin. Each molecule of transferrin can transport two molecules of iron to areas of the body that need this element⁴³. About 60 % of the body's iron is contained in haemoglobin, which is an essential oxygen carrying protein of the blood. Another 30% is stored in ferritin, a protein found throughout the body and a small percentage in myoglobin, a protein specifically utilised by muscles. When body iron stores increase above these relatively normal ratios, proportionally greater amounts of iron are stored in non- blood tissue in ferritin molecules or a complex called haemosiderin³⁷. The body requires iron to make haemoglobin for blood and myoglobin for muscles. Each of these proteins uses iron to supply oxygen and energy for everyday needs. Normally dietary intake offsets daily iron loss which is about 1 to 1.5 milligrams per day. Therefore, one gram of storage of iron is usually adequate to meet all

foreseeable needs. Only small amounts of iron are lost each day through urine and body sweat or as skin cells slough off. The body routinely loses greater amounts of iron as a result of trauma or other conditions resulting in blood loss⁴³. Serum iron concentration measures the amount of ferric iron (Fe^{3+}) bound mainly to serum transferrin but does not include the divalent iron contained in serum as haemoglobin⁴⁴. However serum iron is not a good indicator of iron stores and is not a sensitive measure of iron deficiency, partly because of daily fluctuations. For enhanced utility, serum iron measurements are used in conjunction with TIBC measurements⁴⁴.

Measuring serum ferritin is the best test to evaluate the iron stores. It can be done to detect pre-clinical iron deficiency states. Ferritin levels are low in people who have iron deficiency and are elevated in those with haemochromatosis and other excess iron storage disorders and in those who have had multiple blood transfusions⁴⁴. Ferritin is present in the body in very low concentrations. Plasma ferritin is in equilibrium with body stores, and its concentration declines early in the development of iron deficiency. Low serum ferritin concentrations thus are sensitive indicators of iron deficiency. The generally accepted cut-off level for serum ferritin below which iron stores are considered to be depleted is 15 ng/ml for people aged 5 years and older and 12 ng/ml for people younger than 5 years of age, according to WHO⁴⁴. Ferritin is an acute phase reactant and thus may be increased in people with inflammation, liver disease, chronic infection, autoimmune disorders and some types of cancer. With a few exceptions, including events of inflammation or anaemia of chronic disease, a blood test measuring serum ferritin can provide an accurate surrogate measure of iron stored in organs and non-blood tissue throughout the body⁴³. In infection free situation, serum ferritin is an ideal indicator for diagnosis of iron deficiency⁴⁴.

Total Iron Binding Capacity (TIBC) is most frequently used with serum iron test to evaluate people suspected of having either iron deficiency or iron overload. TIBC measurement indicates the potential capacity of transferrin molecules to bind with serum iron. TIBC measures the amount of circulating transferrin that is available to bind iron⁴⁵. These two tests are used to calculate the transferrin saturation, a more useful indicator of iron status than just iron or TIBC alone. In iron deficiency the iron level is low, but the TIBC is increased, thus transferrin saturation becomes very low. In iron overload states such as haemochromatosis, the iron level will be high and the TIBC will be low or normal, causing the transferrin saturation to increase. When TIBC is at the lower end of the range it is an indication that there is limited capacity for transferrin molecules to accept additional iron. If that occurs in combination with a relatively high measure of serum iron, it is likely that the ability of transferrin to safely bind serum iron is impaired⁴⁵. Iron in plasma that is not bound to transferrin is often called non-transferrin bound iron (NTBI). This is a potentially toxic form of iron that can damage most body systems. Iron toxicity results when circulating iron exceeds the capacity of transferrin available to bind it. This causes oxidative stress, a process that if not countered by the antioxidant defences, will over time result in cell, tissue and DNA damage.

$$\% \text{ Transferrin Saturation} = (\text{serum iron } (\mu\text{mol/L}) / (\text{TIBC } (\mu\text{mol/L})) * 100 \text{ }^{45}$$

Transferrin saturation (TSAT) indicates the percentage of binding sites on transferrin that are occupied by iron and is therefore a measure of circulating iron that is immediately available for erythropoiesis. A reduction in TSAT suggests an inadequate supply of iron to the developing erythrocyte. A higher result is a possible indication of iron overload. Interpretation of the results of iron status markers can be done as follows:

Table 1: Interpretation of iron status markers ⁴⁶

Disease	Iron	TIBC	% Transferrin Saturation	Ferritin
iron deficiency	Low	High	Low	low
Haemachromatosis	High	Low	High	High
Chronic illness	Low	Low	High	Normal/ high
Haemolytic anaemia	High	Normal/low	Low	High
Sideroblastic anaemia	Normal/high	Normal/low	High	High
Iron poisoning	high	Normal	High	normal

1.2 JUSTIFICATION

While many studies have been done on the effect of repeated blood donation on the iron in blood donors in other countries, little is known about the current status in Zimbabwe, a population thought to be iron overloaded ⁴⁷. The thinking behind this project was to try and predict the likelihood of losing a donor. Many people have been allowed to donate and miss on the likelihood of getting iron supplementations thereby getting into the severe complications of iron deficiency anaemia. On the other hand blood has been in short supply in the country for quite a long time now due to a small regular donor base. Some people fear to commit their life to blood donation due to the fear of ‘depleting their own stores.’ Whether

or not this risk is there in our population, which is otherwise thought to be iron overloaded, nobody really knows. Therefore it is the wish of the researchers to find out the iron status in the Zimbabwean blood donor population.

The National Blood Service Zimbabwe (NBSZ) routinely tests blood from donors for transfusion transmissible infections (TTIs). These include HIV 1, HIV 2, Hepatitis B, Hepatitis C and Syphilis. None of these tests give us an indication of the iron status. They also screen their donors using the Copper Sulphate method. This is basically a haemoglobin estimation method. However the use of haemoglobin for screening has been reported to have poor sensitivity. In other studies the Copper Sulphate screening test has been found to erroneously include anaemic people and exclude eligible people, because it does not measure the actual haemoglobin but it is based on the principle of specific gravity^{48, 49}. An accurate diagnosis of a state of iron deficiency requires several laboratory tests, which reflect the iron status of the individual, such as ferritin, TIBC, serum iron and transferrin saturation. It is only after conducting such a thorough study where one can have a clear picture of whether one is due for iron supplementation or can be urged to continue donating. Several studies have also pointed out that iron stores may be depleted in donors with haemoglobin levels above the arbitrary defined limit of anaemia⁵⁰. Therefore donor care can never be complete without doing a thorough study on the iron status of blood donors.

1.3 RESEARCH QUESTION

What is the iron status of Zimbabwean blood donors?

1.4 NULL HYPOTHESIS

Repeated blood donation has no effect on the iron status of blood donors in Zimbabwe.

1.5 OBJECTIVE

To evaluate the iron status of blood donors in Zimbabwe.

1.5.1 Specific Objectives

- To determine the frequency of iron deficiency and reduced iron stores in first time and repeat blood donors
- To determine the effects of repeated blood donation on iron stores in the Zimbabwean donor population.
- To determine the variation of the above with gender

Chapter 2

2.0 METHOD

2.1 STUDY DESIGN

The study was a cross-sectional study in which blood donors who visited the blood donation clinic in August 2013 or attended the Pledge 25 club ladies splash in August 2013 and men's gala in September 2013 in Harare were considered in this study. Only donors who consented were considered in this study. Written informed consent was sought from donors who had passed the Copper sulphate screening test prior to donation. After giving a unit of blood the clinic staff collects two more plain tube samples for blood grouping and transfusion transmissible infection (TTI) testing. Blood is allowed to clot before any testing is done. After the completion of the TTI testing procedure about 1.5 ml of serum was collected into screw-capped serum potties. Serum samples were stored at -20°C pending analysis for serum ferritin, serum iron, and TIBC. Samples were kept in the freezer until the time of analysis. Samples were thawed only once and all the tests were done on the same day. Transferrin saturation was calculated. Donor history was obtained from the laboratory information system (LIS) e-Delphyn.

2.2 Study Setting

2.2.1 Study population

Adult people aged 16 and above donating blood at the NBSZ clinic in Harare and at the Pledge 25 club Ladies Splash and men's gala who had passed the copper sulphate screening test and consented were considered in this study. This included first time donors as well as

repeat donors. First time donors were the baseline or sort of reference point upon which the effects of repeated blood donation were compared.

2.2.2 Sampling frame

Samples for determination of transfusion transmissible infections (TTI) are routinely collected from donors attending the donation clinic. Every sample was identified by a bar code. The researcher collected about 1.5ml of left over samples in serum potties marked by the same barcode number for determination of serum ferritin, serum iron and TIBC from first time donors and repeat donors. The barcode number was the one used to track the donor number of the particular patient as well as the donor demographics and donation history.

2.2.3 Study participants

People attending the blood donation Clinic at NBSZ for routine blood donation and who consented were considered. No extra fee was charged to the participants for the extra iron status tests that were done.

2.2.4 Inclusion criteria

People attending the blood donation Clinic at NBSZ for routine blood donation were considered. Only donors who consented were enrolled into the study.

2.2.5 Exclusion criteria

Donors who have failed the copper sulphate screening test were not considered in this study. Donors who refused to give their consent were also excluded from the study. Donors who had been deferred at any one time during the period under study were excluded.

2.2.6 Study factor

Serum ferritin, iron and TIBC level measurement in blood donors. Transferrin saturation was calculated using the formula:

$$\% \text{ Transferrin Saturation} = (\text{serum iron (umol/L)}) / (\text{TIBC (umol/L)}) * 100$$

2.2.7 Outcome factor

Find the trends in the different categories of blood donors' iron status using the biochemical markers serum iron, ferritin, TIBC and transferrin saturation. Find the prevalence of iron deficiency and iron depletion in first time and repeat blood donors

2.2.8 Sample size

Sample size Calculation

Based on a study by Badar et al (2002), the mean serum iron levels in subjects who have donated blood once (controls) was 16.8 umol/L with a standard deviation of 6.6 and for those who donated blood more than once, mean (SD) of 17.6 (4.3) umol/L. The required sample size to detect a difference in serum iron level between the control and those with more than one blood donation, the minimum required sample size for each is given by;

$$n = \frac{2(Z_{\alpha/2} + Z_{\beta})^2}{\Delta^2}$$

, where $\Delta = (\mu_1 - \mu_2) / \sigma$ is the effect size and $Z_{\beta} = 0.84$ corresponds to 80% power. The minimum required sample size of each group of patients is calculated to be 20 participants. For comparing eight donation groups, the overall required total sample size is 184 participants.

A total of 170 regular (study) and 20 first time (controls) donors were studied. The statistical analysis was done by the ANOVA test

2.2.9 Ethical consideration

The study commenced after approval from the National Blood Service Zimbabwe Research Ethics Committee, Joint Parirenyatwa Hospital and College of Health Sciences Research Ethics Committee (JREC). Study participants were required to sign written informed consent prior to participation in the study (see appendix).

2.2.10 Sample Handling

2.2.10.1 Sample collection and transport

Left over serum samples from the TTI specimens of donors were collected and transported in a cooler box within two hours.

2.2.10.2 Sample storage

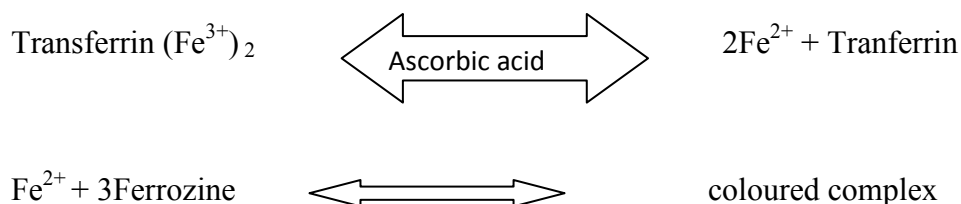
Samples were stored at -20°C at Premier Clinical laboratories until they were analysed.

2.2.11 Assays

Serum iron and ferritin were analysed on the Mindray BS 800 chemistry analyser. The reagents, calibrators and controls were also supplied by Mindray. TIBC was run on the Beckman Coulter CX9. The reagents, calibrators and controls were supplied by the same manufacturer.

2.2.11.1 Iron

Serum iron was analysed on the Mindray BS 800 chemistry analyser based on the principle that under acidic conditions, iron is liberated from transferrin. Ascorbate reduces the released Fe^{3+} ions to Fe^{2+} ions which then react with Ferrozine to form a coloured complex.



The colour intensity is directly proportional to the concentration of iron concentration and can be measured photometrically. The absorbance was measured at 570nm. The precision of the analyser had coefficient of variance (CV) of 0.91% for within-run and 1.3% for between run⁵¹.

2.2.11.2 Ferritin

Serum ferritin was analysed on the Mindray BS 800 chemistry analyser based on the particle-enhanced immunoturbidimetric assay method. When latex bounded with anti-ferritin antibodies reacts with the antigen in the sample, agglutination occurs. This agglutination is detected as an absorbance change which is proportional to ferritin concentration in the sample. The actual concentration is then determined by a calibration curve prepared from calibrators of known concentration. Absorbance was read at 570nm. The precision of the analyser had coefficient of variance of 1.38% for within-run and 0.82% for between run for level 1. For level 2 the CV is 0.70% for within run and 0.61% for between run⁵².

2.2.11.3 TIBC

TIBC was analysed on the Beckman Coulter CX9 analyser. Transferrin in serum is completely saturated by adding excess ferric ion in the form of ferric chloride. Any iron not bound to transferrin is absorbed by aluminium oxide in the column. Iron bound transferrin in the supernatant is measured by the TIBC reagent. TIBC reagent is used to measure the iron concentration by a timed end point method. In the reaction, iron is released from transferrin by acetic acid and is reduced to the ferrous state by hydroxylamine and thioglycolate. The

ferrous iron is immediately complexed with the ferrozine iron reagent. The analyser measures the change in absorbance at 560nm which is directly proportional to the concentration of iron bound to transferrin in the sample and is used by the analyser to calculate and express the TIBC. The precision of the analyser had coefficient of variance of 2.6% for within-run for level 1. For level 2 the CV is 1.6% for within run⁵³.

2.2.11.4 Transferrin Saturation

Transferrin saturation was calculated using the formula

$$\% \text{ Transferrin Saturation} = (\text{serum iron (umol/L)}) / (\text{TIBC (umol/L)}) * 100$$

Chapter 3

3.0 Results

A total of 190 (109 males and 81 females) blood donors participated in this study. These were 170 regular (study) and 20 first time (controls) donors were studied. First time donors can be considered non donors since they would not have donated up until this point. The median age was 23 years and the inter quartile range was 19.0-27 years. The range of the age was from 16 years to 67 years. The median number of donations was 4 and the interquartile range was 5-6 units over the two year period under study.

The median serum iron levels was 13 $\mu\text{mol/L}$ with the inter quartile range from 10.0-17.8 $\mu\text{mol/L}$. For ferritin the median value was 30 ng/ml with the inter quartile range from 18.0-56.8 ng/ml. The median for TIBC was 74.1 $\mu\text{mol/L}$ with the inter quartile range from 63.3-82.3 $\mu\text{mol/L}$. For transferrin saturation the median was 17.6 % with the inter quartile range from 12.0-26.9 %.

Donor characteristics are shown in Table 2 below.

Table 2: Characteristics of blood donors

Characteristic	N=190
Age	23 (19.0-27.0)
Gender	Male
	109 (57.4%)
	Female
	81 (42.6%)
Average number of donations	4 (5-6)
Serum iron ($\mu\text{mol/L}$)	13 (10.0-17.8)
Serum ferritin (ng/ml)	30 (18.0-56.8)
TIBC ($\mu\text{mol/L}$)	74.1 (63.3-82.3)
Transferrin saturation (%)	17.6 (12.0-26.9)

All data: Median (IQR), unless otherwise stated. (IQR- Inter Quartile Range)

Table 2 shows all the donor characteristics such as gender, age, average number of donations as well as the median and inter quartile rang values of the different iron status markers.

Table 3 below summarises the distribution of the blood donors who participated in the study.

Table 3: Distribution of the blood donors in the 8 groups by gender

Group	1	2	3	4	5	6	7	8	Total
Male	10	10	17	15	11	15	14	16	109
Female	10	12	12	11	15	10	11	0	81
Total	20	22	29	26	26	25	25	16	190

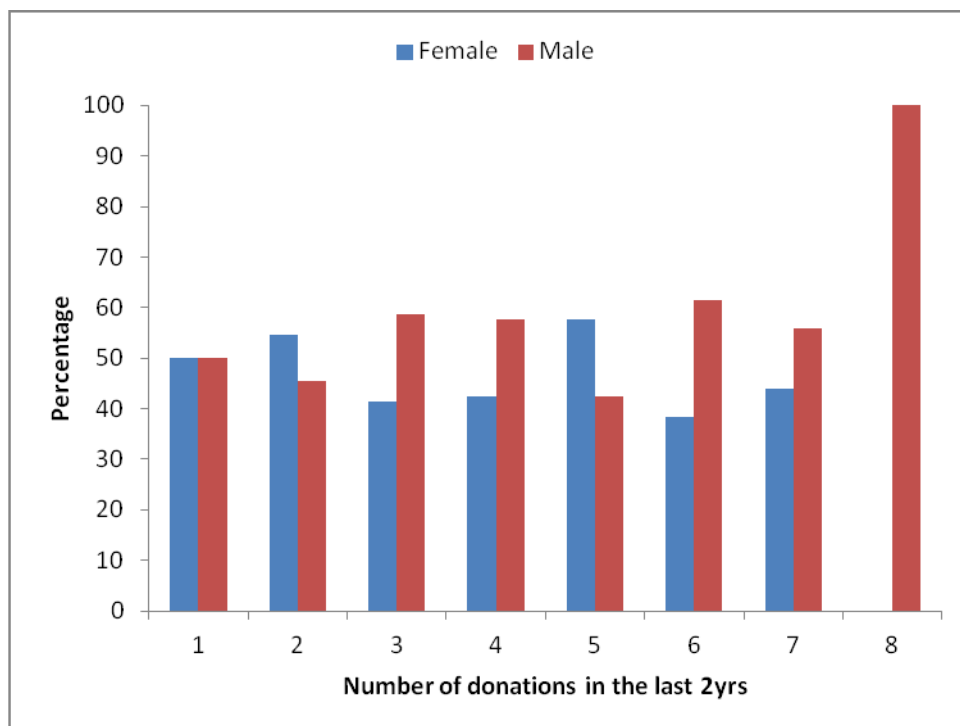


Figure 1: Graph showing the percentage distribution of males and females in the different donor groups

There was statistically significant differences between males and females in all the iron status parameters measured. The p values were < 0.001 , < 0.01 , $= 0.011$ and < 0.01 for serum iron, serum ferritin, TIBC and transferrin saturation respectively. Therefore data was analysed separately for males and females.

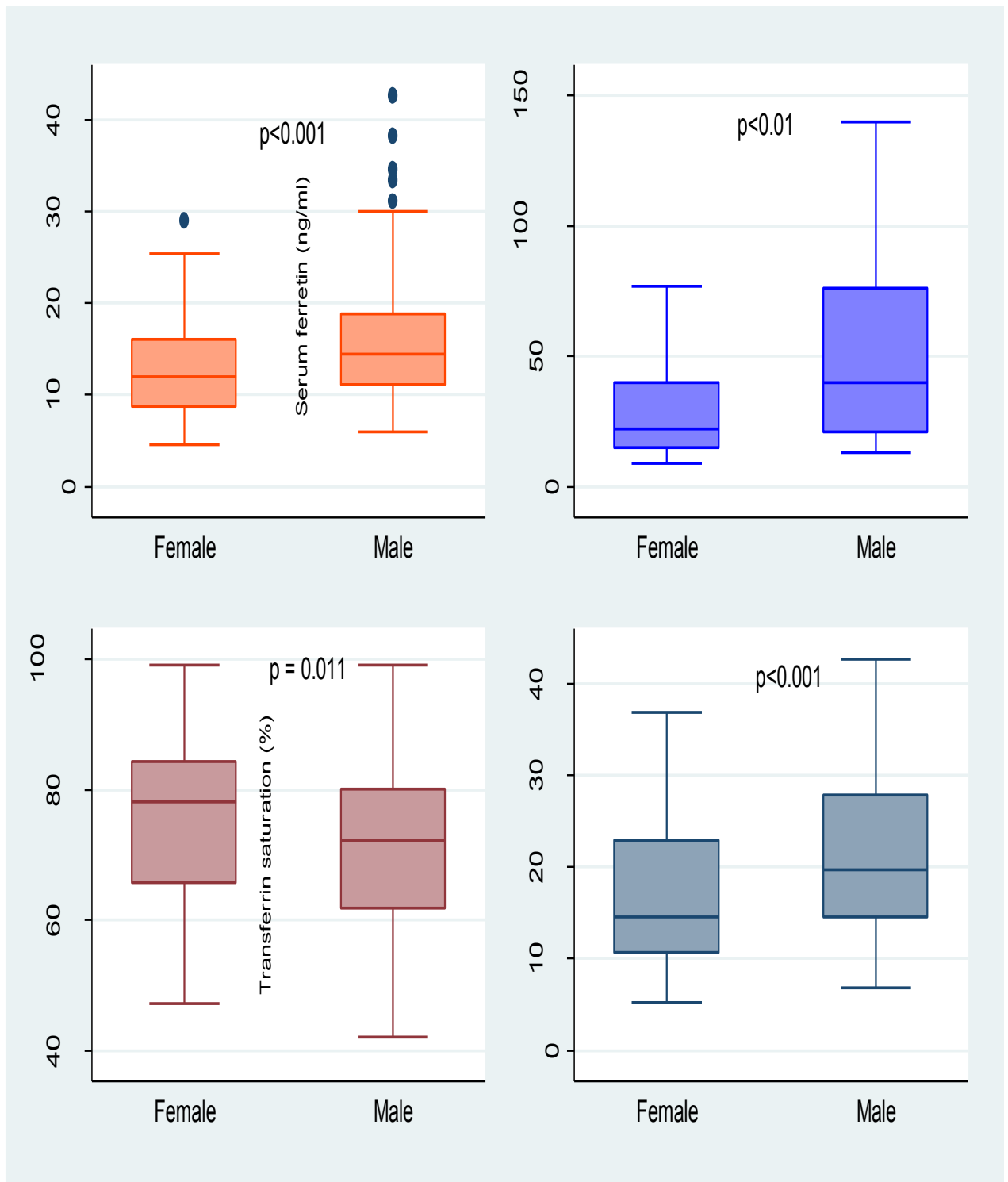


Figure 2: Box and whisker plots for the different iron status markers

The figure above show the diagramatic distribution of the four iron status markers used in the study. Median values as well as the inter quartile ranges are shown as well as outliers as in

the case of serum iron. They show clearly where the majority of study subjects were concentrated.

The statistical analysis was done by the ANOVA test. It was found that there was statistically significant differences in the men's mean iron levels between the control group (group 1) and group 8 (19.73 vs 11.46) ($p = 0.003$). For the other groups there was no difference. For the females there was no statistically significant difference in the serum iron levels. As for serum ferritin levels there was statistically significant differences in the mean ferritin levels between group 1 and groups 6, 7 and 8 ($p < 0.01$) for men. For females there was statistically significant difference in the mean ferritin levels between the control group and group 6 and 7 ($p < 0.05$). For the other groups there was no statistically significant difference.

For TIBC though the general trends showed an increase in the TIBC concentration with an increase in the number of donations and there was no statistically significant difference between the control group and the other groups as far as males were concerned. However for females there was statistically significant differences in the mean serum TIBC levels between the control group and group 7 only (68.3 vs 83.6 $\mu\text{mol/L}$) ($p \text{ value} = 0.0043$). For men there was a statistically significant difference in the mean transferrin saturation between group 1 and group 8, (29.02% vs 15.5%) ($p \text{ value} < 0.001$). For the other groups there was relatively no statistically significant differences. For women there was no statistically significant differences between all the blood donor groups. For example between group 1 and group 8 (23.96% vs 10.5%) ($p \text{ value} = 0.0538$).

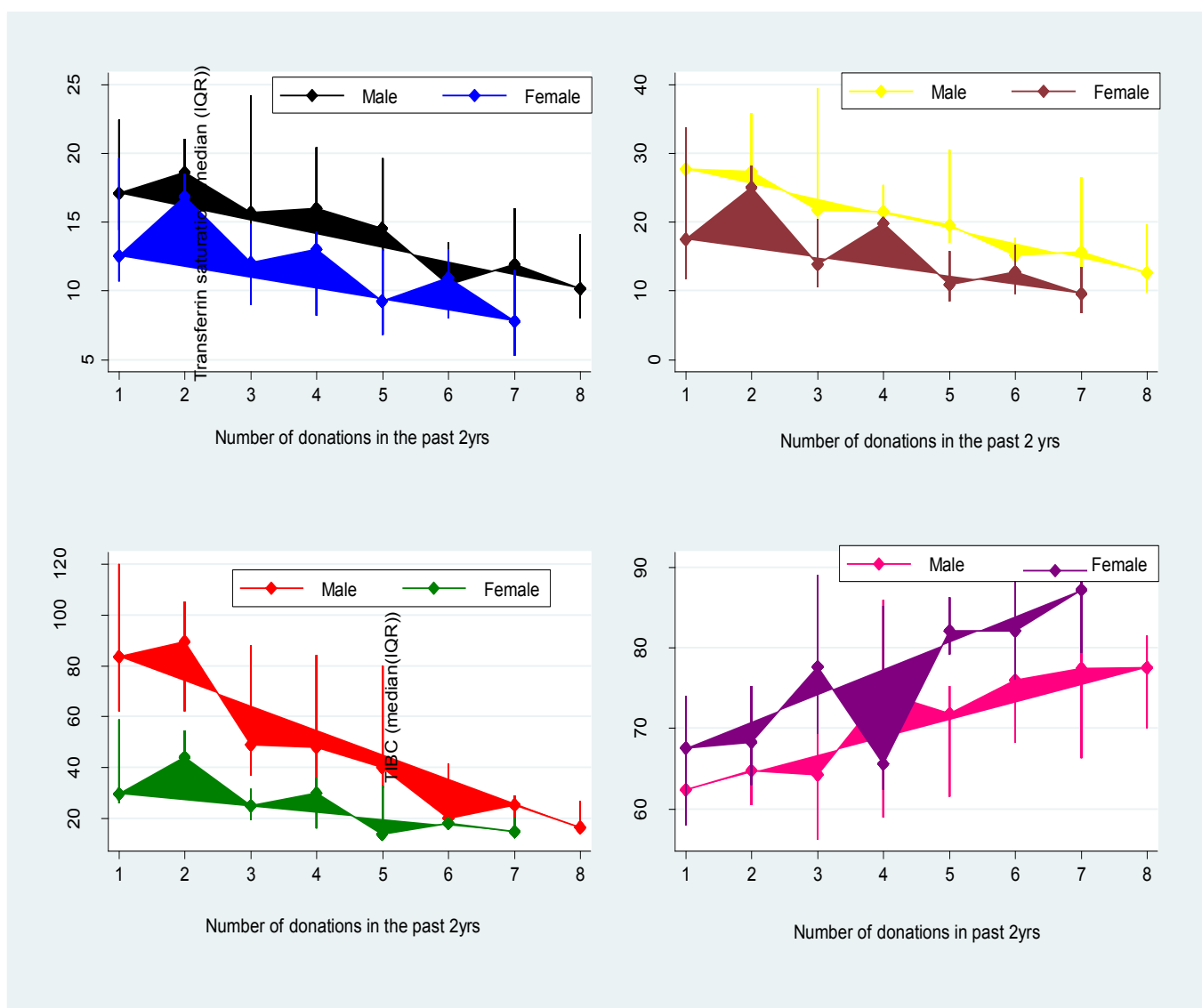


Figure 3: Distribution of the different iron status markers concentration in males and females against the number of donated units in the past 2 years

The units of measurement were: serum iron ($\mu\text{mol/L}$), serum ferritin (ng/ml), TIBC ($\mu\text{mol/L}$), transferrin saturation (%).

Figure 3 clearly shows that there was generally an inverse relationship between serum iron, serum ferritin and transferrin saturation and a direct relationship between TIBC and the number of donations made in the past two years.

Donors were classified as being iron deficient when their ferritin levels were less than 15 ng/ml and transferrin saturation was less than 16 % (WHO 2001)⁴⁴. They were classified as having reduced iron stores when the serum ferritin levels were in the range 15-30 ng/ml. Table 4 below summarises the distribution of people who had reduced iron stores and who had iron deficiency in the respective blood donor groups. Figure 4 shows the distribution of all the people with iron stores of 30 ng/ml and below by their respective percentages in the groups.

Table 4: Distribution of the blood donors with reduced iron stores and iron deficiency in the 8 groups by gender

Group	1	2	3	4	5	6	7	8	Total
Male	0(0)	0(0)	3(0)	4(0)	2(0)	11(0)	10(0)	6(6)	36(6)
Female	6(0)	2(0)	7(2)	5(2)	2(9)	7(1)	6(5)	n/a	35(19)
Total	6(0)	2(0)	10(2)	9(2)	4(9)	18(1)	16(5)	6(6)	71(25)

Key: reduced iron stores (iron deficient)

Females with iron deficiency were 23.45 % and those with reduced iron stores were 43.2 %. For males the figures were 5.5% and 33.0 % for iron deficiency and reduced iron stores respectively. The overall prevalence of iron deficiency in the study population was 13.2% and the prevalence of reduced iron stores was 37.4 %.

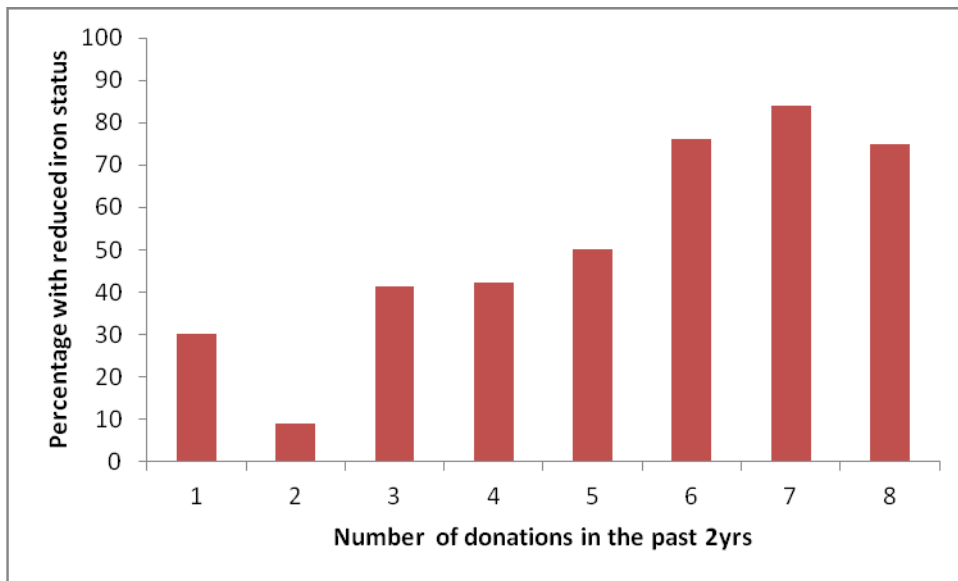


Figure 4: Graph of the total number of people with reduced iron stores per group

The general trend in the bar graph above shows that there is a direct relationship between the reduced iron stores and the number of donations in the past two years. The ratios of males and females in the different donor categories may be responsible for the not so very direct relationship but the general trend shows a direct relationship

Chapter 4

4.0 Discussion and Conclusion

4.1 Discussion

The well-being and health of blood donors is of paramount importance in transfusion medicine and all blood bank policy makers should know this. Blood banks have a responsibility to protect donors and prevent them against developing anaemia¹⁰. Why haemoglobin estimation methods are still the methods of choice in screening blood donors despite calls for many years to include biochemical markers which are more sensitive confuses many. Maybe a study done in our own population was all that the policy makers needed to take the necessary steps. Blood transfusion centres should be focused on maintaining iron balance⁴⁴

In this study statistically significant difference was obtained between serum iron, ferritin, TIBC and transferrin saturation ($p < 0.001$, $p < 0.01$, $p = 0.011$ and $p < 0.001$) respectively between males and females (figure 2). This makes sense as you would expect men and women to have different iron levels. This was the starting point and the reason why most data was analysed separately.

There was a general decrease in the mean serum iron, serum ferritin and transferrin saturation levels and a general increase in the TIBC levels as the number of units increases in the two year period under study even though some parts showed statistically significant differences and others did not. This was similar to several studies done around the world^{32, 54-56}. Abdullah (2011) had the same findings although his study only had serum iron and serum ferritin and other haematological parameters³⁸. Mozaheb et al's findings were consistent with ours in that repeated blood donation had significant effect on the iron balance in all blood donors⁵⁹.

Jeremiah et al also found out that regular donors were adversely affected as shown by reduction in both haematological and biochemical parameters³⁷. Djalali et al also found results similar to ours in that iron status indices were all significantly lower in the subjects than in the controls⁵⁷.

However in a study done in Nigeria Akpotuzor et al found that there was no difference in biochemical iron parameters between male donors and healthy controls⁵⁰. However, it should be noted that the donors were not separated into categories as they were in this study. In another Nigerian study by Adediran et al in 2013 the haemoglobin concentration, packed cell volume, and serum iron levels were found not significantly affected by regular blood donation¹⁰.

The overall prevalence of iron deficiency in the study population was 13.2% and that of reduced iron stores was 37.4 % (table 4). This was almost similar to a Germany research done by Alvarez–Ossorio et al who found figures of 12% and 26% for iron deficiency and reduced iron stores respectively⁵⁴. Nadarajan (2002) also found out an overall prevalence of iron deficiency of 38.4 %⁴⁸. Badar et al (2002) reported much higher prevalence in iron deficiency, with figures as high as 40% for males donating 6 times and 50 % for males donating 7 times in two years¹⁸.

The different figures in the prevalence of iron deficiency and depleted iron stores are due to different geographical locations of the areas where the researches were conducted. Differences in dietary habits, worm infestation, poverty and also the policies of the national blood transfusion services differ. It was also observed that the donor groupings were different. Some researchers put people who donated different number of units in the same group. For example people who donated 2-5 units were all in group III in a study by Abdullah (2011)³⁸. All these factors can account for the differences in figures reported. The prevalence

of anaemia and iron deficiency varies in different populations and no consistent relationship between the two can be applied throughout the world⁹.

Figures may be different from study to study but the general trends show that repeated blood donation results in the reduction of the iron stores of blood donors (figure 3). That is despite the fact that the particular donors would still be having sufficient haemoglobin to pass the various screening tests done by different blood transfusion services. This is a serious call to include the biochemical parameters in the screening and monitoring of blood donors. It can be seen from the graphs above that some donors who are still continuing with the blood donation already have depleted iron stores.

Proper classification of iron deficiency can be difficult because of a lack clear strategy of defining it and also the fact that the reference ranges used are from foreign populations. Local reference ranges would have given a clear picture of the Zimbabwean iron status as compared to the scenario where Zimbabwean donors are compared and classified according to other populations' reference ranges. The bone marrow smear is still the gold standard in the diagnosis of iron deficiency, but it is an invasive method^{41,59}. Ferritin was used for determining the iron deficiency and reduced iron stores because it is the recommended by WHO⁹. Transferrin saturation was also recommended as a better marker of iron status than both TIBC and serum iron. Serum iron is not a sensitive measure of iron deficiency, partly because of daily fluctuations⁴⁴. Low serum ferritin concentrations are sensitive indicators of iron deficiency⁴⁴.

The study had a limitation in that ferritin is an acute phase reactant. Therefore it is elevated in inflammatory conditions. Donors are screened prior to donation to rule out any potential ill effects but still the inclusion of a C-reactive protein (CRP) to rule out elevations of ferritin due to other causes would have helped the interpretation of results better. Though the sample

size was surpassed in this study, researchers still think that a much bigger sample size would give a better reflection of the population of Zimbabwe. It would also be better to decentralise the sample collections to all provinces of the country to have a clearer picture of the iron status of the whole population. Inclusion of the haematological parameters such as haemoglobin would have added a lot of value to the project in that comparison between the chemistry perspective and the haematological side would have been made, thereby clarifying the need to include the biochemistry parameters in the screening process.

4.2 Conclusion

Repeated blood donation causes a reduction in the iron stores of the blood donors in Zimbabwe. Regular donors appear to have reduced iron stores when compared with first time donors. There is need to include biochemical markers, (serum iron, serum ferritin, TIBC and transferrin saturation) in the screening of blood donors.

Recommendations

There is need to determine reference ranges of our own population as those that come with the machines and the ones which are on the different websites refer to different populations from ours. The inclusion of soluble transferrin receptor could add a lot of value as it is a very sensitive marker and highly quantitative marker of iron depletion. It is also an early marker and its proportion increases directly to tissue iron deficit⁶⁰. Blood transfusion centres can also retain a number of donors to checking yearly ferritin levels in frequent blood donors. Checking ferritin levels in first time donors and regularly thereafter could be of great help in the management and monitoring of donors and it can also help in giving advice as to how frequent one should donate. A larger study should be carried out to confirm these preliminary findings.

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Appendices

Appendix 1: English Consent Form

SUBJECT INFORMED CONSENT

PROTOCOL TITLE: The iron status of Zimbabwe blood donor population.

DETAILS OF RESEARCHER: My name is Donald Vhanda. I am studying for an MSc in Clinical Biochemistry at the University of Zimbabwe with the Institute of Continuing Health Education. As part of my study I am required to submit a research project.

PHONE: 0772 957 195

PROJECT DESCRIPTION: I am conducting this research to evaluate the iron status of the Zimbabwe donor population. The study also aims to find out if there is any correlation between the number of units donated and the levels of the iron status markers namely serum ferritin, serum iron, and TIBC.

SIGNIFICANCE OF IRON STATUS: Blood donation results in loss of iron and iron deficiency is the most common cause of anaemia. Donor care can never be complete without knowing their iron status. Whether multiple donations cause iron deficiency or not is the question that necessitated the need to conduct this study, especially in the light that the Zimbabwean population has been found to be iron overloaded.

YOUR RIGHTS: Before you decide whether or not to volunteer for this study you must understand its purpose, how it may help you, any adverse effects and what is expected of you. This process is called informed consent.

PURPOSE OF RESEARCH STUDY: The purpose of this research is to find out the iron status of the Zimbabwean donor population

PROCEDURES INVOLVED IN THIS STUDY: I am going to access your patient records to find out the number of donation you have had to date. Left over sample from routine tests done on your sample is going to be used for the measurement of serum ferritin, serum iron and TIBC (total iron binding capacity) (these are iron status markers).

CONFIDENTIALITY OF RECORDS: There shall be no records kept together of your data and your name so no one will know your results without your consent. You shall be assigned a study number which cannot be linked to your personal information by third parties. All data shall be kept under lock and key in the University of Zimbabwe department of Chemical Pathology for 3 years after which they shall be destroyed.

STUDY WITHDRAWAL: You can choose not to enter the study or withdraw from the study at any time without prejudice or victimisation of any kind.

PROBLEMS/QUESTIONS: Please ask any questions or raise any queries you might have about this study through the investigator D. Vhanda telephone 0772957195 or 04-251 730-2 (extension 229).

AUTHORISATION: I have read and understood this paper about this study or it was read to me. I have understood that being in this study is voluntary and I may opt out at any time. I will get a copy of this consent form. (Initial all the previous pages of this consent form)

Name (print)

Signature

Date

Legal Representative (print)

Signature

Date

Researcher Signature

Date

Appendix 2: Shona Consent Form

CHIBVUMIRANO CHEKUPINDA MUTSVAGIRIDZO

MUSORO WETSVAGIRIDZO

Ongororo yemamiriro e iron muropa revanhu vanogara vachipa ropa muZimbabwe.

ZVAMUNGADE KUZIVA PAMUSORO PEMUNHU ARI KUITA TSVAGIRIDZO

Zita rangu ndinonzi Donald Vhanda. Ndiri mudzidzi weMSc yeClinical Chemistry pachikoro cheUniversity yeZimbabwe. Ndinotarisiwa kuti ndiite tsvagiridzo sechikamu chedzidzo yangu.

NHAMBANDA DZEFONI: 0772 957 195

TSANANGURO PAMUSORO PETSIVAGIRIDZO

Muongororo iyi ndiri kutsvaga kuti ndione kuti iron yakamira sei muropa revanhu vanogara vachipa ropa muZimbabwe. Tsvagiridzo iyi yanga ichida kuongorora kana pane shanduko iripo pamamiriro akaita zve iron muropa kuburikidza nekupiwa kweropa kunoitwa kakawanda kuburikidza nezviratidzo zvinoti izvo che ferritin, che iron, ne che TIBC kune avo vanopa ropa muZimbabwe.

ZVINOBATSIREI KUZIVA MAMIRIRO EZVE IRON: Kupa ropa kunoita kuti iron irasike kubva kumuviri uye kushomeka kwe iron ndicho chikonzero chikuru chekushomeka kweropa muvanhu. Kuchengetedza hutano hwevanopa ropa hakungambokwana pasina kuziva mamiriro ezve iron muvanhu. Zvokuti kupa ropa kazhinji kunokonzera kushomeka kwe iron here kan kwete ndiwo mubvunzo watiri kuda kupindura netsvagurudzo ino tiri muchiedza chokuti vanhu vemuZimbabwe vanoziyikanwa nekuva ne iron yakawanda

KODZERO YAKO

Usati wapinda mutsvagiridzo iyi unofanira kunzwisisa kuti iri kuitirwei, ingakubatsirei, pane njodzi ingakuwirei here uyezve chii chinotarisiwa kubva kwauri. Kana munhu abvuma kupinda mutsvagiridzo nemuitiro uyu ndiyo inonzi chibvumirano chine kunzwisisa.

CHINANGWA CHETSIVAGIRIDZO

Ndiri kuongorora mamiriro akaita iron muropa revanhu vanopa ropa muZimbabwe

NZIRA DZESVAGIRIDZO

Nemvumo yenyu ndichashandisa nhoroondo yekupa kwenyu ropa inowanikwa mumagwaro e National Blood Service Zimbabwe. Pamunotorwa ropa richiongororwa pamunopa ropa ndichatora ropa rinenge rasara ndoongorora huwandu hwe ferritin, iron ne TIBC huri muropa renyu.

CHITSIDZO CHEMAGWARO ENYU

Hapana mashoko pamusoro penyu achachengetwa aine zita renyu pamwe chete. Naizvozvo hapana achagona kuziva kuti ndimi ani asina mvumo yenyu. Vamunotaura navo mutsvagiridzo iyi ndivo chete vachaziva zvakananzika zvenyu zvichabuda mutsvagiridzo iyi. Munguva yetsvagiridzo zvinyorwa zvichagara muchivharira chinenge chichizovhurwa

nemutsvagi chete. Ropa richashandiswa nemuongorori rinenge rakanyorwa nhamba risina zita.

KUBUDA MUTSVAGIRIDZO

Munokwanisa kuramba kupinda mutsvagiridzo kana kubuda chero ipi nguva zvisingakanganise mabatirwo enyu kana marapirwo enyu muchipatara.

MATAMBUDZIKO /MIBVUNZO

Kana muine mibvunzo munogona kubvunza munhu ari kuita tsvagiridzo iyi anonzi D Vhanda iko zvino kana kumubata panhamba dzerunhare dzinoti 0772957195 kana pa04-251730-2 (extension 229).

MVUMO YENYU

Ndaverenga gwaro rino uyezve ndanzwisisa zvarinoreva. Ndinoziva kuti munhu anopinda muongororo iyi nekuda kwake. Ndanzwisisa kuti munhu anogona kubuda muongororo iyi chero ipi nguva pasina chaanorasikirwa nacho. Ndichawana rangu gwaro rechibvumirano ichi kuti ndichengete. (Isai runyoro rwenyu pamapeji ose echinyorwa chino)

Zita

Runyoro

Zuva

Mumiririri

Runyoro

Zuva

Runyoro rwemuongorori

Zuva