"UTILITY OF ESTIMATION OF SERUM FERRITIN AS A SCREENING TEST FOR BLOOD DONORS A CROSS – SECTIONAL STUDY"

By

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Dissertation submitted to the

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In partial fulfillment of the requirements for the award of the degree of

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IN

PATHOLOGY

Under the Guidance of

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Date: **DR. ILA SINGH**

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LIST OF ABBREVATIONS USED

SF	Serum Ferritin
ID	Iron Deficiency
IDA	Iron Deficiency Anemia
Hb	Hemoglobin
WHO	World Health Organisation
MCV	Mean Corpuscular Volume
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
RDW	Red cell Distribution Width
ELISA	Enzyme-Linked Immunosorbent Assay
RNP	Ribonucleoprotein
IRMA	Immunoradiometric Assay
ESR	Erythrocyte Sedimentation Rate
CRP	C-reactive protein
EDTA	Ethylenediaminetetraacetic acid
pI	Isoelectric Point
Fig	Figure

ABSTRACT

INTRODUCTION

Chronic iron deficiency is a well-recognized complication of regular blood donation. With each donation men lose 242 ± 17 mg and women lose 217 ±11 mg of iron.[1] A healthy individual can donate blood up to four times a year, *i.e.*, at three monthly intervals as iron stores get replete within this time period. Cut-off value of hemoglobin of 12.5 g/dl is used for blood donation.[2]

Since hemoglobin levels may be normal in the presence of reduced iron stores, individuals potentially at risk for developing iron deficiency anemia can be detected only by serum ferritin estimation.[1] Ferritin is the main iron storage compound in the body and can therefore be used as a sensitive index to detect the earliest stage of iron deficiency.[2]

OBJECTIVE

A cross-sectional study was carried out on 235 donors who were donating blood voluntarily in the blood bank of BLDEU Shri B.M. Patil Medical College, Hospital and Research centre, Vijayapur.

Study period: 1st November 2013 to 30th June 2015.

MATERIALS

Prospective blood donors who fulfilled standard blood donor selection criteria were included in the study.

Pre-donation hemoglobin assessment was done. Donors were selected based on routine donor selection criteria. After blood donation of 350/450 ml of whole blood, additional samples were collected in plain and EDTA vacutainers and processed. EDTA blood was tested and routine hematological parameters like Hb,

hematocrit, MCV, MCH, MCHC, RDW of the sample were measured. Serum ferritin estimation was done using ELISA technique.

RESULT

Inter-gender and inter-group comparison of serum ferritin was done**.** Among male donors the mean serum ferritin values showed a decreasing trend with increasing number of donations per year and this was statistically significant but the same was not observed with female donors. Hematological parameters such as hemoglobin, hematocrit, MCV, MCH, MCHC and RDW did not show significant fall with increasing number of donations.

CONCLUSION

The present study indicates that SF level estimation in screened blood donors is essential to understand iron store status. Therefore, it may be introduced as a screening test before donating blood especially in repeat blood donors even though their haemoglobin level is normal.

KEY WORDS: Serum ferritin, voluntary donors, repeat blood donors, hemoglobin.

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INTRODUCTION

Chronic iron deficiency is a well-recognized complication of regular blood donation. With each donation men lose 242 ± 17 mg and women lose 217 ±11 mg of iron. A healthy individual can donate blood up to four times a year, *i.e.*, at three monthly intervals as iron stores get repleted within this time period.[1] Cut-off value of hemoglobin of 12.5 g/dl is used for blood donation.[2]

Increasing donation frequency has been shown to be associated with a distinct fall in iron stores.[3] In majority of blood banks, hemoglobin and/or hematocrit measurements are used as routine screening tests for allowing a person to donate blood.[2, 4] This easy and inexpensive approach has been considered to be a reasonably good method of protecting donors against the development of progressive iron deficiency anemia. However, such routine methods do not reflect on the status of the total body iron content of an individual which decrease with increasing frequency of blood donation as stated earlier.[2] Since hemoglobin levels may be normal in the presence of reduced iron stores, individuals potentially at risk for developing iron deficiency anemia can be detected only by serum ferritin estimation.[1] Therefore iron status of the donors needs to be identified and necessary steps for iron supplementation need to be taken.[1,5]

Ferritin is the main iron storage compound in the body and is present mainly in the reticulo-endothelial cells of liver, spleen and bone marrow. A small amount of ferritin (31-300µg/l) is normally found in the circulating plasma and provides a precise quantitative measure of the total iron in the storage compartment and can therefore be used as a sensitive index to detect the earliest stage of iron deficiency.[2]

Iron stores are classified as depleted, when serum ferritin (SF) value is < 14 µg/l; reduced, when value ranges between 15-30µg/l; normal or replete, at value

between 31-300 µg/l and increased, keeping values beyond 300µg/l. Iron deficiency anemia is considered to be present when SF is < 12 µg/l.[6]

Hence, a more logical and specific test which can identify the iron store status of the body like SF level in blood has been included.

AIMS AND OBJECTIVES

To assess the utility of estimation of serum ferritin levels as a screening test in voluntary blood donors.

REVIEW OF LITERATURE

Blood can save millions of life, and young people are the hope and future of a safe blood supply in the world. No transfusion service can survive without blood donors. Since the 1980s blood collectors worldwide have focused on two central themes: blood product safety and an adequate blood supply. But this achievement has come at a price: iron depletion of the repeat blood donor.[7]

The voluntary unpaid blood donation is a humanitarian act towards the sick by the healthy hence, the wellbeing and health of the blood donors is of prime importance for the medical profession. A lot of money is being spent for the screening of donors for protection of recipients but very little attention is given to the health status of donors. The main reason is the fear of losing the donor in a time when the demand of blood is high all over the world and the donors are becoming scarce. [8]

The need for blood is great. On any given day, approximately 32,000 units of Red Blood Cells are needed. Accident victims, people undergoing surgery, and patients receiving treatment for leukaemia, cancer, or other diseases, such as sickle cell disease and thalassemia, all utilize blood. More than 23 million units of blood components are transfused every year.[8, 9]

A donor generally donates approximately 450 ml blood at the time of donation. One gram of haemoglobin contains 3.4 mg of iron. In a normal individual with 15 g of haemoglobin per dl, 100 ml of blood contains approximately 50 mg of iron. Thus removal of only 2 ml of blood results in the loss of 1 mg of iron. If 450 ml of blood are taken in a donation approximately 225 mg of iron will be lost. If the donor has no iron deficiency, the erythrocytes and the haemoglobin level will generally return to normal within 3–4 weeks. Hence adequate iron stores are very

important in maintenance of the donor health. The process of blood donor selection is designed to ensure that the donation does not cause harm to the donors.[8]

The only known significant disadvantage of blood donation is the potential risk for iron deficiency. [7]

Therefore, acceptable frequency of donation is normally two or three times a year. After a single donation, a person needs approximately 3 months to replenish iron stores.[8]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. [11]

Iron is a vitally important element in human metabolism. It has a central role in erythropoiesis and is also involved in many other intracellular processes in all the tissues of the body. [7]

Iron is a universal cofactor for mitochondrial energy generation and supports the growth and differentiation of all cell types. The regulation of systemic iron is through the proteins 'Transferrin' (iron mobilization) and 'Ferritin' (iron sequestration). The physiologic importance of the storage iron is that it provides a rapidly available supply in the event of blood loss.[10]

Serum ferritin concentration is an indicator of mobilizable body iron stores. In various clinical disorders serum ferritin is found to correlate with the marrow iron stores assessed histologically. Serum ferritin averages about 90 and 30 microgram/L in normal men and women respectively, a difference that accurately reflects the well-known difference in iron stores of the two sexes. [8]

The iron content of the body is kept constant by maintaining a balance between the amount absorbed and the amount lost. The iron available in the diet is absorbed in a small portion only. This amount also depends upon the interaction of

foods, drugs and abnormal components of diet. Iron requirements depend on age, sex, race, pregnancy, lactation and altitude.[8]

Considerations of the iron deficiency have traditionally focused on anemia, that reduces maximum Oxygen consumption and maximum work performance in proportion to its severity. Whatever the basis, iron deficiency induces a hypochromic microcytic anemia. Simultaneously depletion of essential iron containing enzymes in cells throughout the body may cause other changes, including koilonychia, alopecia, atrophic changes in the tongue and gastric mucosa, and intestinal malabsorption. It is now appreciated that depletion of iron dependent tissue enzymes occurs in concert with the decrease in haemoglobin production. Most importantly there is now evidence that iron deficiency has an adverse effect on brain function. Lack of iron effects the body systems and produces variable symptoms. Increased catecholamine levels in children leading to abnormal behaviour have been found associated with iron deficiency. In a study, an impaired response of triiodothyronine to cold was reported in iron deficiency.[8]

Therefore it seems reasonable to secure adequate iron reserves in the donor population in order to maintain an appropriate donation potential and to avoid possible non-hematological side-effects of iron deficiency, i.e. changes in immune function, energy metabolism and work performance.[3]

For blood collection an appropriate hemoglobin screening method should be available so as to accept as many suitable donors as possible and to prevent any inappropriate deferrals.[4]

An anemic person cannot donate blood. To protect against this possibility, a haemoglobin test, usually done by the ‘copper sulphate specific gravity method’ is required for every blood donor on each donation. The American Association of blood

banks has a standard of minimum haemoglobin of 13.5 g/dl for men and 12.5 g/dl for women donors. However, except for a very few modern blood banks most of the blood banks in our country do not care much for the donors health. [8]

Women of child bearing age are especially liable to iron depletion; many men can donate more frequently without such an ill effect. Men are able to maintain adequate iron status while donating up to 5 units of blood annually, but women are at risk for iron deficiency if they donate more than 1 unit per year. Iron stores are exhausted in virtually all female donors regardless of the frequency of blood donations. [8]

The potentiality of the individual donor to give blood without developing ID and iron deficiency anemia (IDA) varies widely, probably due to differences in nutritional iron intake, the differences in prevalence of ID in each study population, menstrual iron loss in females, the frequency of blood donation and the use of supplemental iron. [7]

The clinical implications of ID and IDA are not insignificant, including fatigue, reduced work performance and intellectual capacity, reduced endurance, restless leg syndrome, pica, and cognitive and immune function changes. The degree of symptomatology is proportionate to the severity of the anemia.[7,12,13]

Moreover, low hemoglobin (Hb) accounts for 4-10% of total deferrals, with the vast majority occurring in women. Therefore it seems reasonable to secure adequate iron reserves in the donor population in order to maintain an appropriate donation potentiality and to avoid possible hematological and non-hematological complications related to ID.[7,12,13]

However, we know that IDA is the last stage of ID and it is evident that Hb measurement, alone, is inadequate to detect blood donors with ID without anemia. It

is not surprising that the current practice results in accepting many iron-depleted female donors who have normal Hb values.[13]This calls the need for a more accurate control of Hb measurement techniques i.e. assessing serum ferritin (SF) levels.[7]

Today 62 countries have blood transfusion services based entirely on voluntary blood donation, up from 39 in 2002. India reports the greatest increase in the number of voluntary unpaid blood donations from 3.6 million in 2007 to 4.6 million in 2008.[14]

In 2008, 162 countries provided data to WHO on 91.8 million donations. The data comes from countries that account for a total 6.2 billion people, representing 92% of the global population.[14]

The frequency of iron deficiency was positively associated with the frequency of donations ($p< 0.001$). To address this issue few studies have been undertaken. The study conducted by Garg *et al*[2] included 116 donors .Predonation haemoglobin , haemogram and serum ferritin were done. They were divided into two groups on the basis of number of donations. First time donors *(81.07±97.12)* had higher mean serum ferritin level than those in repeat donors *(46.01±49.09)*.10.52% of first time donors and 27.5% of repeat blood donors were found to be iron deficient as indicated by serum ferritin level <12 ng/ml.In addition a higher RBC count, reticulocyte % and lower MCV were noted in repeat donors. They concluded that haemoglobin estimation was not adequate to detect iron deficient non anaemic state in repeat blood donors. Serum ferritin proved to be a better investigation to detect the same and they proposed that estimation of serum ferritin should be done in repeat donors. They also recommended iron supplementation for an adequate period post donation.

The study conducted by Mittal *et al* [1] included 400 voluntary blood donors . These donors were divided into four groups depending upon their periodicity of blood

donations. Pre-donation haemoglobin assessment was done by copper sulphate method. Serum ferritin was estimated by indirect ELISA. In this study, it was observed that the number of female donors with deficient iron stores was more as compared to male donors. First time donors had higher mean serum ferritin levels than that in repeat donors. The frequency of donations per year was more predictive of decreased iron stores rather than the number of lifetime donations. An increase in donation frequency was accompanied by a significant decrease in serum ferritin; values<15 μg/l were found in 21 and 46 per cent of male and female donors respectively who donated once per year, in 29 and 27 per cent in those who donated twice per year and in 49 and 100 per cent in those who donated thrice per year. They concluded that haemoglobin estimation alone in regular blood donors may not be adequate; serum ferritin estimations may need to be done to detect pre-clinical iron deficiency states. They also suggested iron supplementation to be considered in regular, repeat voluntary blood donors.

The study conducted by Cancado *et al* [3] in sao Paulo ,Brazil included three hundred blood donors. It was observed that the frequency of iron deficiency in blood donors was 11.0%, of whom 5.5% (13/237) were male and 31.7% (20/63) female donors. The frequency of iron deficiency was higher in multi-time blood donors than in first-time blood donors, for male blood donors (7.6% versus 0.0 %,) and female ones (41.5% versus 18.5%,). The frequency of iron deficiency found was higher among the male blood donors with three or more donations per year and among the female blood donors with two or more donations per year. They concluded that blood donation is a very important factor for iron deficiency in blood donors, particularly in multi-time donors and especially in female donors. The high frequency of blood donors with iron deficiency found in this study suggested a need for a more accurate

laboratory test, as hemoglobin or hematocrit measurement alone is not sufficient for detecting and excluding blood donors with iron deficiency without anemia.

Boulton *et al* [9] measured serum ferritin concentrations in regular donors and found that 8% of men and 38% of women had reduced iron stores after 2 donations in 1 year.

In all the studies the most pronounced influence of donation frequency was on serum ferritin concentrations in men and older women. MCH and MCV fell, but the medians stayed within the normal ranges.[9]

IRON DEFICIENCY IN FREQUENT AND FIRST TIME FEMALE BLOOD DONORS

About 28% of women in the reproductive age who donate just two times per year suffer from iron deficiency.[16] At each blood donation, approximately 213 mg iron or 9% of the total iron stores in women are lost. If the lost iron stores in these donors are not replenished and they continue to donate blood, it results in iron deficiency anaemia and deferral of these donors in the future.[16, 17]

Thus, a considerable number of regular donors who at present are the best source of safe blood are lost. In fact, iron deficiency anaemia is the main limiting factor in regular donors. [17]

Study done by M. Boulahriss and N. Benchemsi [16] showed haemoglobin concentration, serum ferritin, serum iron were significantly lower in frequent female blood donors when compared with the results of same parameters in first time female blood donors. The results show that the frequency of iron deficiency in frequent female blood donors is 43% and in the first time female blood donors is 14%.

SOME BRIEFING ABOUT SERUM FERRITIN

It consists of a protein shell with a molecular mass of about 500 kDa composed of 24 subunits. The protein shell encloses a core of ferric-hydroxy-phosphate which can hold up to 4000 atoms of iron.[18]

GENETICS

A range of isoferritins is found in various human tissues. These are composed of combinations of two types of subunit, H and L [19].

The expressed gene for the H-subunit is on chromosome 11 at 11q13 and that for the L-subunit is on chromosome 19 at 19q13-ter. There are multiple copies of the ferritin genes.[18, 20]

Human H and L genes have a similar structure. The H-subunit is slightly larger than the L-subunit (178 amino acids compared with 174 amino acids) but on electrophoresis in polyacrylamide gels under denaturing conditions the apparent differences in relative molecular mass are rather greater (21 kDa and 19 kDa). Human H and L sequences are only 55% homologous whereas the degree of homology between L-subunits and H-subunits from different species is of the order of 85% .[18, 21]

STRUCTURE

A ferritin subunit has five helices and a long inter-helical loop. The loop L and the N-terminal residues are on the outside of the assembled molecule of 24 subunits. The C-terminal residues are within the shell. H and L chains adopt the same conformation within the molecule. In human tissues H-rich isoferritins (isoelectric point (pI) 4.5–5.0) are found in heart muscle, red blood cells, lymphocytes, monocytes, HeLa cells and other, but not all, cultured cells [18, 22]

L-rich isoferritins are more basic (pI 5.0–5.7) than H-rich isoferritins and are found in the liver, spleen and placenta. The pI of ferritin is not significantly affected

by its iron content, which varies from tissue to tissue and with the tissue iron content.[18]

HAEMOSIDERIN

Ferritin is a soluble protein but is degraded to insoluble haemosiderin which accumulates in lysosomes. Both ferritin and haemosiderin provide a store of iron that is available for protein and haem synthesis. Normally much of the stored iron in the body (about 1 g in men and less in pre-menstrual women and children) is present as ferritin, but during iron overload the proportion present as haemosiderin increases.[18]

REGULATION OF FERRITIN SYNTHESIS AND BREAKDOWN

Ferritin synthesis is induced by administering iron. The initial response of apoferritin synthesis is regulated by translation rather than transcription. This requires the movement of stored mRNA from the ribonucleoprotein fraction (RNP) to the polysomes followed by an increased rate of translation of ferritin subunits. This response is the same for H and L subunits.[18]

FUNCTIONS RELATED TO IRON STORAGE

The major function of ferritin is to provide a store of iron which may be used for haem synthesis when required. Iron uptake in vitro requires an oxidizing agent, and iron release requires a reducing agent [21].

There are differences in the rate of iron uptake between apoferritins with varying proportions of H and L-subunits with H-rich isoferritins having the highest rate of iron uptake in vitro. Such isoferritins are found in cells which either have a high requirement for iron for haem synthesis, such as nucleated red cells and cardiac muscle, or which do not appear to be involved in iron storage, such as lymphocytes. In the tissues where iron is stored, ferritin contains mostly L-subunits seen in liver and spleen. [18]

Recent studies with recombinant H24 and L24 molecules have demonstrated that the ferroxidase activity of ferritin is a property of the H-subunit and that L24 molecules have little ability to catalyse iron uptake. Iron storage therefore seems to require ferritin that is rich in L-subunits.[21]

PLASMA (SERUM) FERRITIN

With the development of a sensitive immunoradiometric assay (IRMA) detection of ferritin became possible in the serum or plasma of normal individuals.

Relationship to storage iron levels : Serum ferritin concentrations are normally within the range 15–300 µg/l and are lower in children than adults. Mean values are lower in women before the menopause than in men, reflecting women's lower iron stores caused by the losses during menstruation and childbirth. The changes in serum ferritin concentration during development from birth to old age reflect changes in the amounts of iron stored in tissues. [23]There is a good correlation between serum ferritin concentration and storage iron mobilized as a result of phlebotomy. This suggests a close relationship between the total amount of stored iron and the serum ferritin concentration in normal individuals. Serum ferritin concentration decreases with blood donation and increases with alcohol intake.[24] In women after the menopause the ferritin concentration increases but remains lower than in men.[18]

The serum ferritin concentration is relatively stable in healthy persons. In patients with iron deficiency anemia, the serum ferritin concentration is typically less than 12–15 µg/l. This threshold has been established in a number of studies by determining the serum ferritin concentrations of patients with iron deficiency anemia and a reduction in the level of reticuloendothelial iron stores is the only common cause of a low serum ferritin concentration. This is the key to the use of the serum ferritin assay in clinical practice [25].

SERUM FERRITIN IN VARIOUS DISEASES

Serum ferritin in acute and chronic disease: The acute phase refers to a series of events that occur in response to infection or tissue damage. The local reaction is termed inflammation and the systemic response is referred as the acute phase response. The acute phase response may be induced by toxic chemicals, physical trauma, infection, inflammation, malignancy, tissue necrosis (e.g. myocardial infarction) and immunization. The clinical and metabolic features of the acute phase response include fever, leucocytosis, thrombocytosis and metabolic alterations, as well as changes in the concentration of a number of plasma proteins. In anemia of chronic disease the most important factor controlling serum ferritin concentration is the level of storage iron. However the serum ferritin concentration is higher than in patients with similar levels of storage iron but without infection and inflammation. Inflammation causes a rapid drop in serum iron concentration which may be due to an increase in apoferritin synthesis which inhibits the release of iron to the plasma [26].

In few studies there was 3 fold rise in serum ferritin concentration after acute infection with the maximum concentration reaching within 1 week.[18] A ferritin concentration of <15 µg/l indicates the absence of storage iron while concentrations >100 µg/l indicate the presence of storage iron. Serum ferritin in the range of 15–100 µg/l is difficult to interpret. It would seem logical to combine the assay of serum ferritin with a measure of disease severity such as the ESR or the concentration of CRP. In assessing of the adequacy of iron stores to replenish haemoglobin, the degree of anaemia must also be considered. Thus a patient with a haemoglobin concentration of 100 g/l may benefit from iron therapy if the serum ferritin concentration is below 100µg/l.[18]

Serum ferritin and liver disease: The other major influence confounding the use of the serum ferritin concentration to estimate iron stores is liver disease. The liver contains much of the iron stored in the body, and any process that damage liver cells will release ferritin. It is also possible that liver damage may interfere with clearance of ferritin from the circulation. In patients with liver damage a low serum ferritin concentration always indicates absent iron stores, a normal concentration indicates absent or normal iron stores but rules out iron overload, whereas a high concentration may indicate either normal or high iron stores and further investigation may be necessary to distinguish between the two. [18]

Serum ferritin concentration and malignancy: A high concentration of ferritin is seen in most patients with pancreatic carcinoma, lung cancer, hepatoma and neuroblastoma. Patients with acute leukaemia generally have a higher serum ferritin concentration than normal but this is not the case for patients with chronic leukaemia. In Hodgkin's disease the concentration of ferritin increases with the stage of disease, but is not related to the histological type of disease. It is likely that the high concentration of ferritin in the serum in malignancy is due to an increase in the concentration of storage iron, to liver damage, or to inflammation, as well as a consequence of the direct release of ferritin from the tumors. Whatever the cause is, the result is an increase in the concentration of L-rich isoferrritin in the serum rather than accumulation of 'tumour-specific' isoferritins.[18]

Exceedingly high serum ferritin concentrations: are seen in liver necrosis, in adult-onset Still's disease and in patients with acquired immunodeficiency syndrome (AIDS) having a reactive haemophagocytosis syndrome. [18]

High serum ferritin concentrations and congenital cataract: An interesting cause of a high ferritin concentration in the absence of iron overload is associated with inherited cataract formation.[18]

RED CELL FERRITIN:

The ferritin concentration declines throughout the process of cell maturation and only about 10ng/cell (10-18 g/cell) remains in the erythrocyte. Red cell ferritin concentration has generally been measured with antibodies to L-ferritin and reflects the iron supply to the erythroid marrow. The concentration tends to vary inversely with the red cell protoporphyrin concentration. The red cell ferritin concentration does not therefore necessarily indicate the concentration of iron in storage. An assay for red cell ferritin has seen little routine application because it is necessary to have fresh blood in order to separate the red from white cells, which have a much higher ferritin concentration.[18]

ASSAY OF SERUM FERRITIN:

Samples in many assay both plasma and serum gives the same results but in some cases plasma collected in EDTA gives different values to serum. Samples may be stored at - 20°C or -80 °C for several years. Several rounds of freezing and thawing do not lead to changes in serum ferritin concentration, nevertheless freezing and thawing should be kept to a minimum.[18]

PITFALLS IN ASSESMENT OF FERRITIN ASSAYS:

There are a number of theoretical and practical problems associated with the assay of serum ferritin. Theoretically, this is because ferritin consists of a family of isoferritins. Practically this has not been a problem because, in general, the ferritin found circulating in the plasma is similar to the L-rich ferritin found in liver or spleen.[18]

A more practical concern is the very wide range in ferritin concentration that can be encountered in serum. One of the effect is called the “high-dose hook” effect (such as in necrosis of the liver) seen in early two-site immunoradiometric assays. In this situation a very high ferritin concentration could give readings in the lower part of the standard curve .[18]

Some methods give low recoveries of ferritin from plasma collected in EDTA, and the use of the plasma samples should therefore be investigated carefully.[27] Another problem could be because of antibodies to some animal proteins which are sometimes present in human serum. These can interfere with the assay of serum ferritin, giving a spuriously high ferritin concentration.[18]

METHODOLOGICAL AND BIOLOGICAL VARIABILITY OF MEASURES OF IRON STATUS

Assays of blood for indicators of iron status vary greatly in both methodological and biological stability. The more complicated procedures involved in immunoassays lead to a greater variation in ferritin assays, with a coefficient of variation of at least 5%. There is however little evidence of any significant diurnal variation in serum ferritin concentration. There is no information on seasonal factors influencing most of these analyses. The effect of menstruation on serum ferritin (SF) concentration was lowest for women whose blood was drawn during their menses and highest for women examined in the luteal or late luteal phase of their menstrual hence, cyclical variations could be a potential source of error [28]. That’s why female donors who were menstruating were deferred.

Variability was less in venous serum as compared to capillary serum. Starvation or even fasting for a short period can cause an increase in the serum ferritin

concentration while a vitamin C deficiency may reduce the ferritin concentration. Moderate exercise has little effect on serum ferritin concentration.[18]

THE HAEMOGLOBIN LEVELS IN DONORS

Blood donors are required to have a hemoglobin level of at least 12.5 g/dL or hematocrit of 38% in order to donate blood. This is to ensure that donors have an adequate number of red blood cells (RBCs) for donation as well as adequate iron stores for erythropoiesis following donation.[29]

DONATING BLOOD CAN CAUSE IRON DEFICIENCY

A healthy blood donor loses about 200-250 mg of iron per blood donation, constituting a roughly 6% in men and 9% in women with an average loss of 4.0g and 2.5g total body iron, respectively.[30] A double RBC donation, permitted every 16 weeks, results in the loss of up to 500 mg. The body compensates for this loss by mobilizing iron stores in the form of ferritin.[31]

For this reason, mean ferritin levels are significantly lower in blood donors than in non-donors and studies have shown that iron stores decline with frequent blood donation.[31] Men usually have the most dramatic drop in ferritin levels because of higher iron stores before donation. After 6-8 phlebotomies the ferritin level is about 40% lower than at baseline.[31]The proportion of male donors with decreased iron stores went from 8 to 19% with an increase from 5 to 6 donations per year.[31]

HEMOGLOBIN SCREENING METHODS

There is no consensus among blood banks on the best method for blood donor anemia screening.[32]In hospitals and laboratories, the gold standard for hemoglobin detection is the hemoglobincyanide method provided by automatic hematology analyzers.[33]

Screening tests for potential blood donors however require quicker, easier, and more cost-effective testing methods that do not require a venipuncture.

The tests which are commonly used for primary screening are as follows,

1. Copper sulfate method
2. Microhematocrit method
3. Hemacue method
4. Automated hematology analyzer

Though the first three tests used for hemoglobin estimation are quick, easy, and relatively inexpensive, their sensitivity, specificity, and accuracy are lower than that of automatic hematology analyzers.[33]These tests are discussed below in detail.

***CuSO4* (COPPER SULFATE) METHOD**

This is a qualitative screening test based on specific gravity. The density of the drop of blood is directly proportional to the amount of hemoglobin it contains. The sample of donor's blood dropped into copper sulfate solution becomes encased in a sac of copper proteinase, which prevents any change in the specific gravity for about 15 seconds. If the hemoglobin is equal to or more than 12.5 gm/dL the drop will sink within 15 seconds and the donor is accepted.[34]

If the blood drop sinks to the middle and remains or starts to rise, a microhematocrit or comparable test is usually used to confirm the deferral. This is not a quantitative test and will only show that the hemoglobin is equal to, below, or above acceptable limits. Test results that indicate satisfactory hemoglobin levels are usually accurate, but some results that indicate low hemoglobin levels can be false. Repeating the test by a second method is sometimes used as confirmation.[34]

MICROHEMATOCRIT METHOD

Microhematocrit is a method for rapid determination of hematocrit done on an extremely small quantity of blood (one capillary tube of approximately 10 µL) by use of a capillary tube and a high-speed centrifuge. This method is a little more time consuming than other methods. Microhematocrit is often used to confirm failures with the CuSO4 method. A recent study shows a relatively poor correlation of the microhematocrit with the automated hematology analyzer. Anemia screening using this method failed to detect 35.7% of truly anemic donors.[34]

HEMOCUE METHOD

Some blood centers currently use portable equipment that is able to spectrophotometrically determine hemoglobin. These devices use a 10 µL capillary blood sample to determine hemoglobin by measuring the absorbance of azide methhemoglobin, using a cuvette containing a dry reagent system and a dual wavelength photometer. There was a relatively poor correlation of HemoCue 201(+) with the automated hematology analyzer. However, this method was more accurate (56%) in detecting anemia in prospective female blood donors than the microhematocrit method.[34] The HemoCue 201(+) and the microhematocrit method were equivalent in their donor deferral rate.[34]

EFFECT OF WHOLE BLOOD DONATION ON THE IRON STATUS

Blood donation poses a risk of iron deficiency to blood donors. Iron is an important element of the Hb protein, which is found in red blood cells. In humans, most iron is found in red blood cells, incorporated in Hb. Red blood cells contain about 60-75% of the total body iron. In adults, the total body iron content is normally 3-5 g with typically higher values in men than in women.[35]

With a blood donation, a substantial amount of iron is lost. A donation of 500 ml whole blood contains about 200-250 mg iron, which is 4-8% of total body iron. When the iron intake is not sufficient to replenish the iron loss due to donation, a negative iron balance occurs. Subsequent blood donations may then gradually lead to iron deficiency.[36]

In one study there was a significant correlation between the frequency of donations and serum ferritin levels. The mean ferritin level decreased tremendously in regular blood donors as early as after the first 10 donations and remained stable after 20 or more donations.[30]

Three stages of iron deficiency can be distinguished: iron depletion, iron deficient erythropoiesis and iron deficiency anemia.[37]

Iron depletion is marked by running out iron stores. In the stage of iron deficient erythropoiesis, the iron supply to the erythropoietic bone marrow is becoming insufficient for erythropoiesis. However, Hb levels are still normal. When finally the iron supply becomes insufficient to produce a normal amount of Hb, iron deficiency anemia becomes apparent. Iron depletion and iron deficient erythropoiesis are thus sub-clinical stages; with anemia clinical symptoms appear. The primary function of Hb is oxygen transport through the bloodstream from the lungs to all other tissues in the body, and one of the first symptoms of anemia is decreased fitness through a diminished oxygen supply to the body tissues. Furthermore, as iron is also an important element of several other proteins, iron deficiency also affects DNA synthesis,[38,39] the immune system[40] and energy metabolism through impaired mitochondrial electron transport.[41,42]

Blood donors on average need several weeks to replenish the lost iron after a blood donation.[43] However, there are wide variations in the duration of the recovery

period among individual donors. The European guidelines with relation to a minimum donation interval and a maximum number of donations per year may therefore not be safe for each individual donor. Indeed, depleted iron stores in blood donors are not uncommon[44]and also iron deficient erythropoiesis occurs.[45, 46]

IRON SUPPLEMENTATION POST DONATION

Every blood donation (450 ± 25 mL) is associated with significant iron loss, approximately 200 to 230 mg and the lost iron is not readily replenished. Even with iron-rich diets and excellent compliance, six months or longer are necessary to positively impact SF levels. Therefore, this practice is inadequate in the scenario of blood donation.[3] Recent studies in blood donors have shown that short-term (4- to 8-week course) use of oral iron supplementation at 100-300 mg daily of elemental iron (and even utilizing lower doses such as 20-40 mg/day), is effective in improving Hb levels, in replacing iron loss after blood donation even in menstruating females, in maintaining SF concentrations in a range of 50 to 80 µg/L and significantly reducing blood donation deferral. Thus, low-dose iron administered (100 mg/day) for up to 60 days post-donation appears to be a sound and feasible strategy.[47, 48]

MATERIALS AND METHODS

Source of data

A cross-sectional study was carried out on blood donors who were donating blood voluntarily in blood bank of BLDEU Shri B.M. Patil Medical College, Hospital and Research Centre, Vijayapur.

Study period: 1st November 2013 to 30th June 2015.

Inclusion criteria: Prospective blood donors who fulfilled standard blood donor selection criteria were included in the study.

Exclusion criteria: Prospective blood donors who did not fulfill the above mentioned criteria were excluded from the study.

7.2 Methods of collection of data.

- The study was carried out on donors who come to donate blood voluntarily in blood donation camps conducted by the institute.
- All donors were required to fill voluntary donor selection/rejection form and then general physical examination was done.
- Pre-donation hemoglobin assessment was done.
- Donors were selected based on routine donor selection criteria.
- After blood donation of 350/450 ml of whole blood, additional samples were collected in plain and EDTA vacutainers.
- The samples were processed within 4-6 hours of collection.
- EDTA blood was tested using the 5 part differentiated automated Hematoanalyzer and routine haematological parameters like Hb, haematocrit, red cell count, MCV, MCH, MCHC, RDW of the sample were measured.
- Serum ferritin estimation was done using ELISA technique.

FIGURE 1: HEMATOLOGY ANALYSER SYSMEX XN-1000:

Courtesey- Shri B. M. Patil Medical College, Pathology Lab

Sample Size:

Considering the average frequency of decreased iron stores as 29% (i.e. 8% in males and 50% in females) at the time of blood donation[5] at 95% confidence interval and 20% allowable error the sample size is 235

Statistical formula $n = \frac{(1.96)^2 \, p \times q}{L^2}$

Where p : prevalence rate

q : p - 1

L: allowable error

The calculated sample size is 235

Hence, minimum of 235 cases will be included in the study.

Statistical analysis:

1. Data will be presented as Mean+/- SD.
2. Student's t test.
3. Diagrammatic representation of the data.
4. Correlation coefficient (if necessary).

RESULTS

After taking informed consent of 235 donors, they were divided into 2 groups: Group A: (n- 133) comprised of blood donors who were donating for the first time in which 80 were male donors and 53 were female donors. Group B: (n-102) comprised of repeat donors who had previously donated blood on one or more occasions and had 91 male donors and 11 female donors. Group B was further divided into 3 groups – group I, II, and III. Group B I: (n- 54) comprised of donors who had donated once in the previous year and were donating for the second time. Out of total number of 54 donors, 46 were male and 8 were female. Group B II: (n- 17) comprised of donors who had donated twice in the previous year and were donating for the third time. Out of total number of 17 donors, 16 were male donors and 1 was female donor. Group B III: (n- 31) donors who had donated thrice in the previous year and were donating for the fourth time or more than fourth times. Out of total number of 31 donors, 29 were male and 2 were female. All the donors had hemoglobin value >12.5gm/ dl.

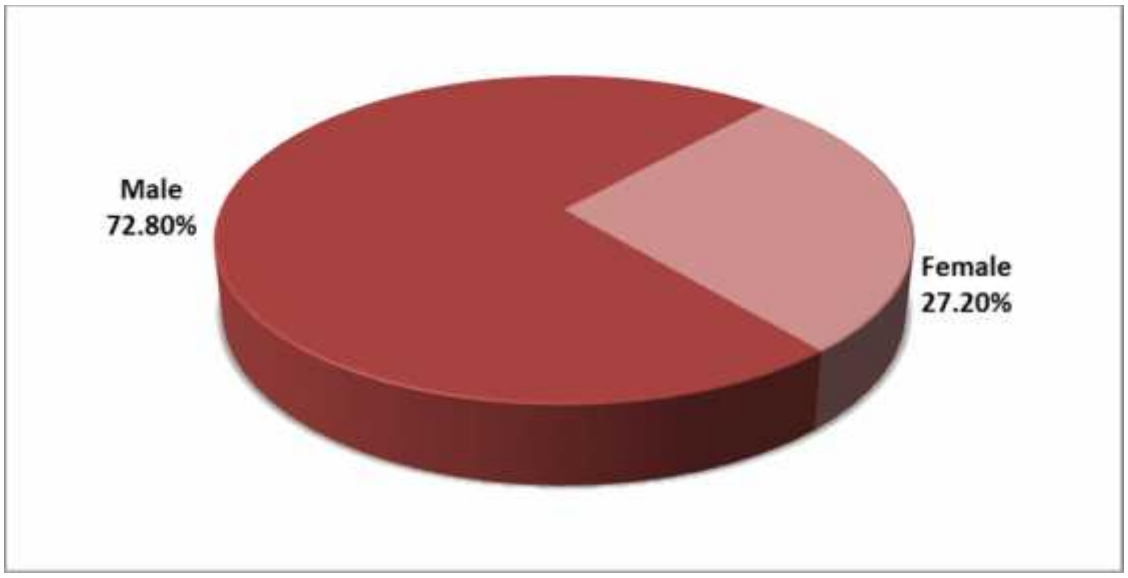
Table 1: Gender and group wise distribution of donors:

		Male	Female	Total
Group A	**1st time**	**80**	**53**	**133**
Group B		**91**	**11**	**102**
Group BI	**2nd time**	**46**	**8**	**54**
Group BII	**3rd time**	**16**	**1**	**17**
Group BIII	**≥4th time**	**29**	**2**	**31**
Total		**171**	**64**	**235**

Table 2: Gender wise percentage distribution:

Gender	N	Percentage (%)
Male	171	72.8
Female	64	27.2
Total	235	100.0

Figure 2: Gender wise percentage distribution:

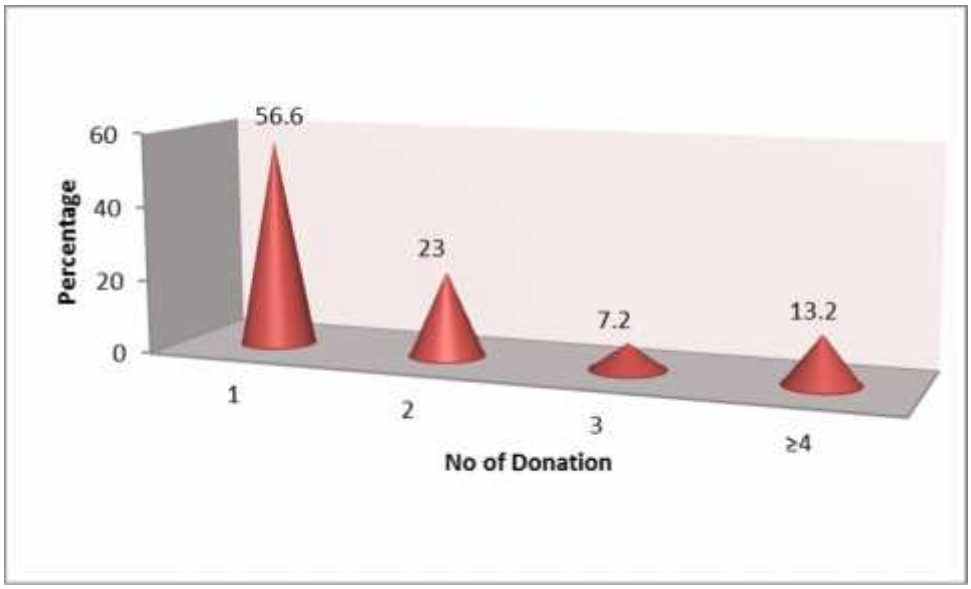


Out of 235 donors who participated, 171 were male donors contributing 72.8 % of the sample size and 64 were female donors comprising 27.2 % of the total donors who participated.

Table 3: Percentage Distribution based on the number of Donations:

No of Donations	N	Percent
1	133	56.6
2	54	23
3	17	7.2
=ñ4	31	13.2
Total	235	100

Figure 3: Percentage distribution based on the number of donations:



The percentage of first time donors donating blood was the highest (56.6%) whereas donors who were donating for the third time comprised only 7.2% of the total donor size.

The mean age of gr A and gr B was similar; the respective values were 25.1±10.4 and 26.2±8.5.

Table 4: Comparison of mean hematological parameters between group A and B among male donors:

Parameters	(A)First time donor (N=80)		(B)Repeat donor (N=91)		p value
	Mean	SD	Mean	SD	
Hb (g/dl)	14.5	1.4	14.8	2.0	0.343
PCV (%)	42.9	3.9	44.5	6.1	0.057
SF (ng/ml)	80.3	91.6	49.1	43.7	**0.004***
MCV (fl)	86.2	11.0	87.5	6.9	0.357
MCH (pg)	29.5	2.7	29.2	3.0	0.557
MCHC (%)	33.8	1.3	33.2	1.4	**0.002***
RDW (%)	14.0	4.1	13.8	1.4	0.600

Note *: significant as $p<0.05$

On comparison of various hematological parameters between group A and group B male donors, it was observed that hemoglobin level in group A was 14.5±1.4 gm./dl and in group B was 14.8±2 gm/dl which was statistically not significant ($p >0.05$).

Also no significant difference was noted between two groups of male donors for parameters like hematocrit (PCV), MCV, MCH, and RDW.

However, the MCHC values also showed a statistically significant difference ($p=0.002$) though in the normal range with group A and group B male donors having values 33.8±1.3 and 33.2±1.4 pg., respectively.

Group A and group B male donors were compared for the serum ferritin values. The mean of SF values for male donors of group A (who were donating for the first time) and group B (who were donating for more than first time) was 80.3±91.6 ng/ml and 49.1±43.7 ng/ml respectively and this difference was found to be statistically significant ($p= 0.004$).

Table 5: Comparison of mean parameters between group A and B among female donors:

Parameters	(A)First time donor (N=53)		(B)Repeat donor (N=11)		p value
	Mean	SD	Mean	SD	
Hb (g/dl)	13.6	1.3	13.2	0.8	0.377
PCV (%)	40.8	4.2	39.1	2.6	0.190
SF (ng/ml)	33.4	33.3	18.8	17.7	0.163
MCV (fl)	85.6	6.8	84.1	6.3	0.506
MCH (pg.)	28.8	2.7	28.3	1.9	0.549
MCHC (%)	33.3	1.1	33.7	0.8	0.259
RDW (%)	14.1	3.3	13.8	2.0	0.735

Note *: significant as $p<0.05$

On comparison of various hematological parameters between group A and group B female donors, it was observed that **NONE** of the hematological parameters including mean SF values showed statistically significant reduction. The mean of SF values for female donors of group A (who were donating for the first time) and group B (who were donating for more than first time) was 33.4±33.3 ng/ml and 18.8±17.7 ng/ml respectively and this difference was found to be statistically insignificant (p value > 0.05). This could be because the serum ferritin levels in women did not fall as much with multiple donations as it did in men. This difference may be misleading because only a small percentage of female donors comprising only 27.2 % of the total donors participated in the study and very few women donated blood more than twice a year.

Figure 4: Comparison of mean SF between group A & B among male and female donors

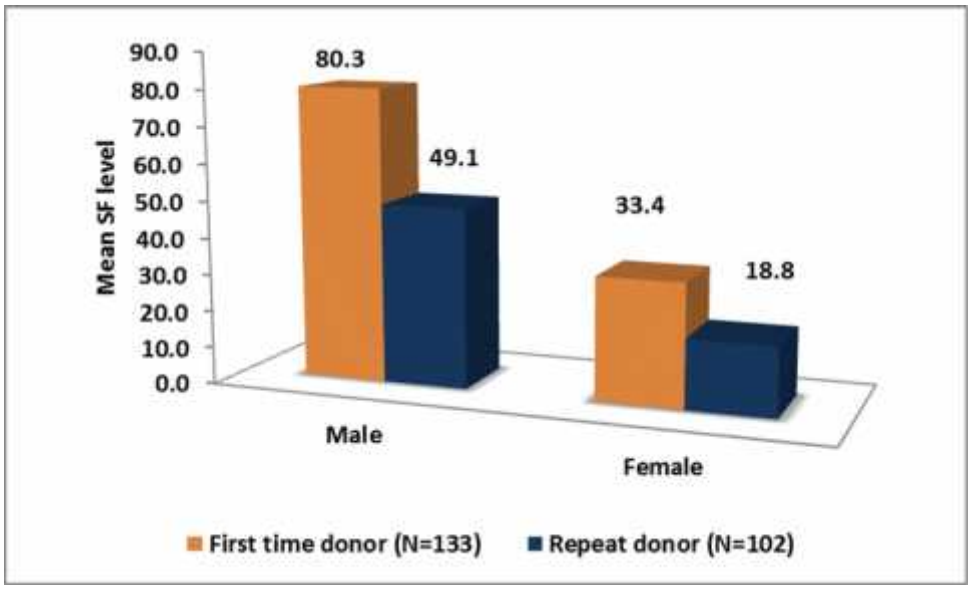


Table 6: Inter-group comparison of mean values of serum ferritin (only those groups showing significant difference are included):

Inter-group comparison between male donors		Mean	SD	Mean	SD	p value
group-A (1st time)	**group-BIII (ñ 4th time)**	80.3	91.6	42.7	39.0	**<0.05***
group-BII(3rd time)	**group-BIII (ñ4th time)**	49.2	56.1	42.7	39.0	**<0.05***

Note *: significant as p<0.05

Inter-group calculation of mean of SF values was done for male and female donors. Among male donors the mean serum ferritin values showed a decreasing trend with increasing number of donations per year. There was a significant mean decrease (P<0.05) in mean serum ferritin values after three blood donations done in the previous years, the values being 42.7 ng/ml vs. 80.3 ng/ml in those donors who came for the first time and had no prior blood donation. Also when mean of SF of group BII donors who were donating blood for the 3rd time (49.2±56.1 ng/ml) was

compared with Group BIII donors (42.7±39 ng/ml) who were donating for the ≥4th time, the reduction in SF was found to be statistically significant.

When inter-group comparison of mean of serum ferritin values was done for female donors it was not found to be statistically significant.

Table 7: Inter-gender comparison of mean SF (ng/ml) levels based on the number of donations:

Groups/No of Donations	Male			Female			p value
	N	Mean SF	SD	N	Mean SF	SD	
(gp-A) 1st time	80	80.3	91.6	53	33.4	33.3	0.001*
(gp-BI) 2nd time	46	53.0	42.2	8	20.0	18.0	0.035*
(gp-BII) 3rd time	16	49.2	56.1	1	5.2	NA	0.458
(gp-BIII) ñ 4th time	29	42.7	39.0	2	20.9	25.7	0.444

Note *: p significant as p<0.05

Comparison of mean SF levels between male and female donors based on the number of times of donation was made. The reduction in mean SF level in group A (donating for the 1st time) and group BI (donating for the 2nd time) female donors was found to be significant as compared to group A and group BI male donors.

Whereas the reduction in mean SF level in group BII (donating for the 3rd time) and group BIII (donating for ≥4th time) female donors was not significant as compared to group BII and group BIII male donors.

This observation may be due to low initial serum ferritin values in female donors which could be due to the nutritional factors superimposed over monthly menstrual blood loss and child birth. Below is a bar diagram depicting the same (Fig: 4)

Figure 5: Inter-gender comparison of mean SF (ng/ml) levels based on the number of donations

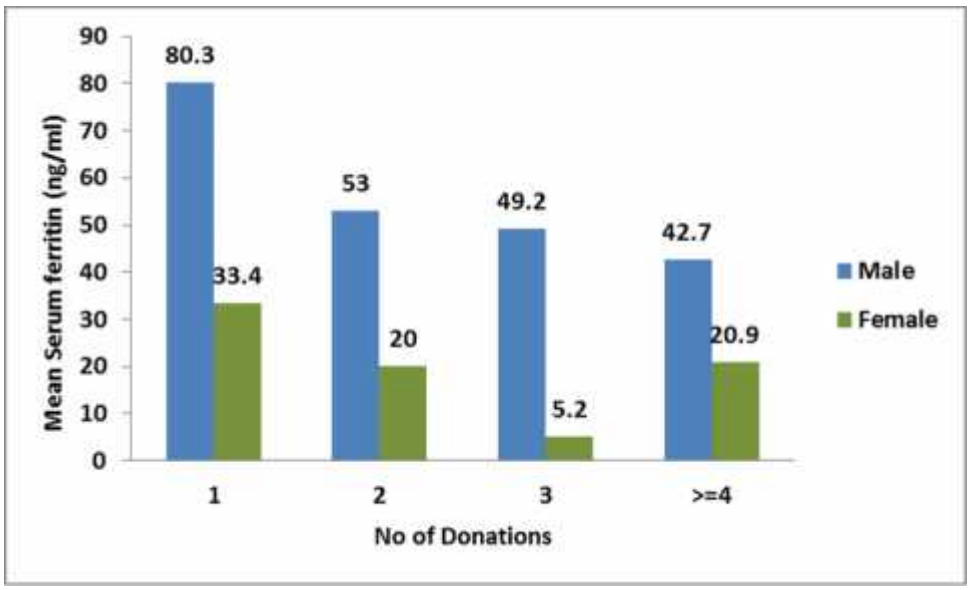


Table 8: Classification of iron stores:

Serum ferritin (ng./ml)	Classification of iron stores
<12(%)	Iron deficiency anaemia
12-14(%)	Iron depleted
15-30(%)	Iron reduced
31-300(%)	Normal or iron replete
>300(%)	Increased iron

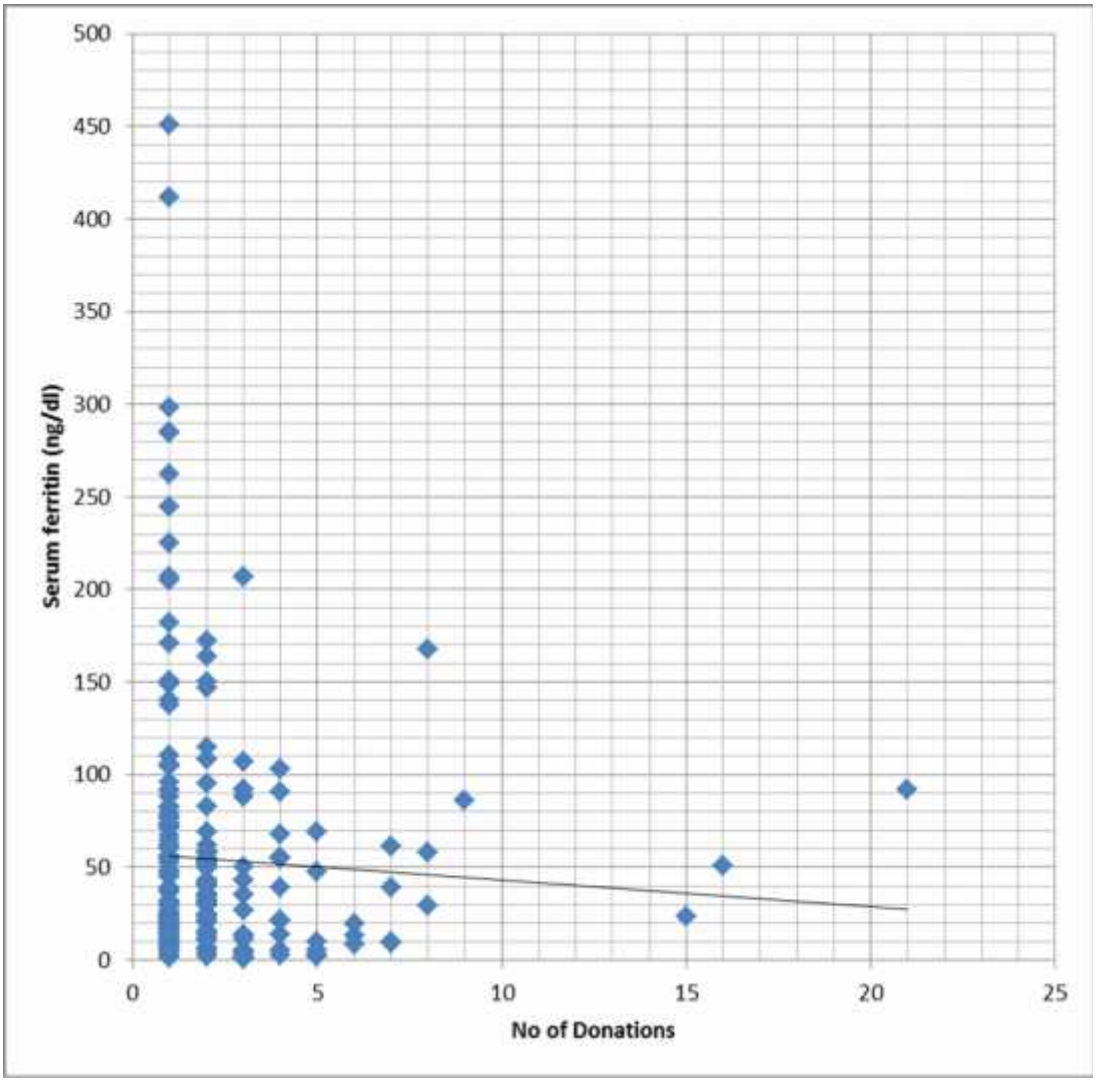
Table 9: Percentage Distribution of donors in groups based on the serum ferritin level

No of Donations	Serum ferritin (ng/ml)				
	<12(%)	**12-14(%)**	**15-30(%)**	**31-300(%)**	**>300(%)**
group-A (1st time)	18(14)	8(6)	44(33)	61(46)	2(2)
group –BI(2nd time)	8(15)	3(6)	9(17)	34(63)	0(0)
group –BII(3rd time)	**6(35)**	2(12)	1(6)	8(47)	0(0)
group –BIII(ñ4th time)	**9(29)**	2(6)	5(16)	15(48)	0(0)
Total	41(17)	15(6)	59(25)	118(50)	2(1)

Also the percentage of donors with iron deficiency anemia as classified by iron stores was higher in group BII (3rd time donors) and group BIII (≥4th time donors) i.e. 35% and 29% respectively as compared to group A which was 14% only. Group A had two donors who were having increased iron as classified by iron stores.

A scatter-gram was plotted for searching any relationship between number of blood donations and SF level.

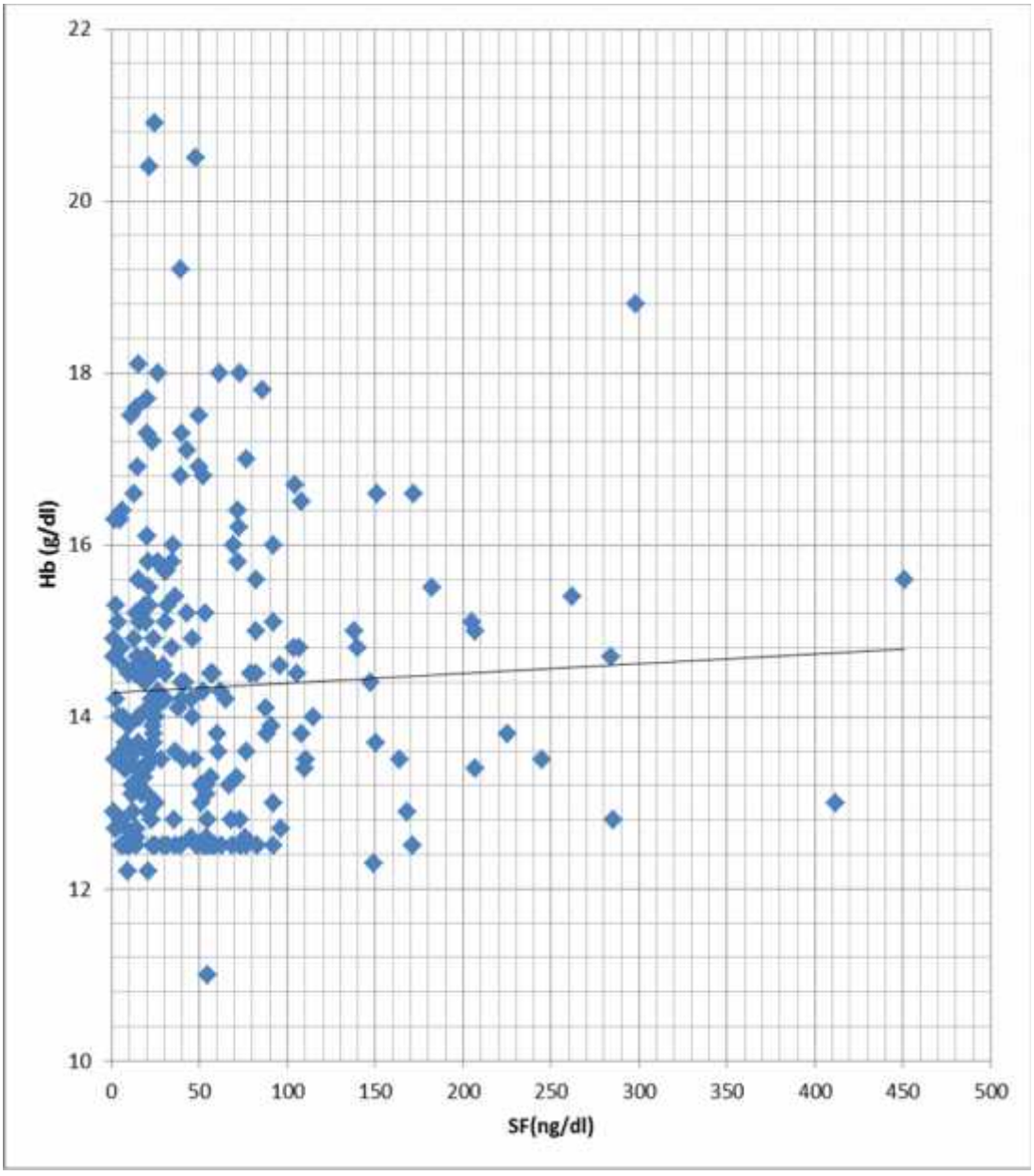
Figure 6: Correlation between SF levels and number of donations:



As depicted in the scatter-gram, a negative correlation was seen between the number of donations and SF level (r= -0.117). Although the SF values of earlier donations were not known, it has been observed that there was a fall in SF value with the increase in the number of blood donations.

Further a scatter-gram was also plotted for searching any relationship between the levels of SF and hemoglobin. When both values have been placed in scatter-gram it was found that there was no correlation between these two observations (r= -0.044).

Figure 7: Correlation between SF levels and hemoglobin:



DISCUSSION

Hemoglobin assessment is an important criterion for blood donor selection. The minimal hemoglobin cutoff is set at 12.5 gm %, which is done to ensure both donor safety and appropriate hemoglobin content in the donated unit. A healthy blood donor loses about 200–250 mg of iron per unit of blood donated which constitutes an average iron loss of 4.0 g and 2.5 g of total body iron in men and women respectively.[30] The body compensates for this loss by mobilizing iron stores in the form of ferritin that correlates well with studies showing decline in iron stores with repeated blood donation. This explains why the mean ferritin levels are significantly lower in blood donors than in non-donors.[49]

There is no consensus among blood banks on the best method for blood donor anemia screening.[50] In hospitals and laboratories; the gold standard for hemoglobin estimation is the use of automated hematology analyzer.

Screening tests for potential blood donors, however, require quicker, easier, and more cost-effective testing methods that do not require a venipuncture and cause minimal discomfort to the donor. Three tests that are commonly used for primary screening are copper sulphate specific gravity method, Hemocue and Microhematocrit. Although these tests are quick, easy, and relatively inexpensive, their sensitivity, specificity, and accuracy are lower than that of an automated hematology analyzer.[50]That is why at our center, we used copper sulfate as primary screening methods, but the results were ultimately confirmed by running the EDTA venous sample of the subject on an automated analyzer.

Safe donor selection is the first step towards safe transfusion services. Various efforts are going on to ensure safe blood supply through screening, education and

strict criteria laid down by the Directorate General of Health Sciences, Ministry of Health and Family Welfare (2003).[51]

Since hemoglobin levels may be normal in the presence of decreasing iron stores, individuals potentially at risk for developing iron deficiency anemia may be detected by serum ferritin estimation.

The aim of the present study is to find out the utility of estimation of serum ferritin in the early detection of falling iron stores in potential donors and to ensure safe donor selection.

Table 10: Mean serum ferritin levels (ng/ml) in different studies based on number of donations in male donors:

Studies	First time donors	Repeat donors	p value
Garg *et al*[2]	81.0±97.1	46.0±49.0	**<0.05***
Jacob *et al* [52]	55.3±36.9	22.0±17.4	**<0.05***
Norashikin *et al*[53]	90.7±66.6	62.0±39.8 (>5 times)	**<0.05***
Nadarajan *et al*[54]	94.2±89.9	47.8±44.3	**<0.05***
Abdul Aziz *et al* [55]	129.5±72.1	78.5±70.2	**<0.05***
Lim *et al*[56]	109.4±77.9	97.2±74.3	>0.05
Abdullah *et al*[6]	131.4±NA	72.4±NA	**<0.05***
Present study	80.3±91.6	49.1±43.7	**<0.05***

P* value <0.05 is significant

As compared to the present study, similar studies were carried out. But the study did by Garg *et al*[2] and Abdul Aziz *et al* [55]correlates well with the present study. In all the studies including the present study there was statistically significant reduction in serum ferritin with an increase in the number of donations. Other such studies done by Jacob *et al*[52], Norashikin *et al*[53], Abdullah *et al*[6] showed similar

findings. Studies done by Nadarajan *et al*[54]and Lim *et al*[56] included male and female donors for comparing the mean of serum ferritin values while in the present study it seemed logical to calculate and compare the values gender wise.

Nevertheless, all the authors concluded that hemoglobin estimation per se was not adequate to detect iron deficient non anemic state in repeat blood donors and proposed that estimation of serum ferritin should be done in repeat donors. They also recommended iron supplementation for an adequate period post donation. In addition to the above findings, Jacob *et al* [52] also found that normal man who was consuming a mixed diet containing about 15 mg of iron daily and losing blood at a rate of 164 ± 34 ml/month did not increase their iron absorption sufficiently to compensate for the iron loss. This further explains why there is a need to review the guidelines on donor acceptance.

Table 11: Mean serum ferritin levels (ng/ml) in different studies based on the number of donations in female donors:

Studies	First time donors	Repeat donors	p value
Present study	33.4±33.3	18.8±17.7	>0.05
Abdul Aziz *et al* [55]	52.5±34.7	36.1±33.0	<0.05*
Nadarajan *et al*[54]	94.2±89.9	47.8±44.3	**<0.05***
Boulahriss *et al* [16]	32.0±20.0	10.0±8.0	**<0.05***
Milman *et al*[57]	24±NA	19.0±NA	**<0.05***
Lim *et al*[56]	109.4±77.9	97.2±74.3	>0.05

P * value <0.05 is significant

At each blood donation, approximately 213 mg iron which constitutes 9% of the total iron stores in women are lost.[16] Study done by Lim *et al*[56] showed results similar to the present study that is, the mean of serum ferritin values which did not fall significantly with multiple donations, but Lim *et al*[56] did not have gender wise intergroup comparison in their study. They did so because no significant difference was demonstrated in male and female donors in developing iron deficiency with increasing number of donations. Whereas in the present study, the serum ferritin levels done separately in women did not fall as much with multiple donations as it did in men. This difference may be misleading because very few women donated blood more than twice a year. **Percentage distribution of male and female donors in various studies is given in table 12.**

Study done by Abdul Aziz *et al* [55], Nadarajan *et al*[54], Boulahriss *et al* [16] and Milman *et al*[57] showed different observations. There was significant fall in serum ferritin with the number of donations as it was in male donors.

Table 12: Percentage distribution of male and female donors in various studies:

Studies	Mittal *et al*[1]	Cançado *et al*[3]	Tondon *et al*[4]	Present study
Male	81%	79%	92%	72.8%
Female	19%	21%	8%	27.2%

In the present study as well as studies done by Mittal *et al*[1], Cançado *et al*[3], Tondon *et al*[4], it was observed that there is inequality between the population of male and female blood donors. Percentage of female donors was much less as compared to the male donors. According to WHO Blood Safety, 2014 data; women contribute only 30% of blood donations globally. Reasons for these gender differences could be prevalence of iron deficiency and pernicious anemia, a higher rate of adverse reactions to blood donation related to body weight (e.g. dizziness, fainting), low body weight among prospective female donors, low level of education, erroneous beliefs and cultural factors among women. These can result in deferral of prospective female donors at the blood donation centers.[58]

Table 13: Mean serum ferritin levels (ng/ml) in different studies based on number of donations in male donors:

Studies	1st time	2nd time	3rd time	≥4th time	p* value was significant after
Present study	80.3±91.6	53.0±42.2	49.2±56.1	42.7±39.0	≥4th time D
Abdul Aziz *et al* [55]	129.5± 72.1	129.1±95.2	82.2±54.5	73.3±87.2	>1st time D
Mittal *et al*[1]	55.6±51.4	44.9±48.6	34.4±37.1	21.4±25.4	>1st time D
Badar *et al*[8]	74.4±22.0	68.1±20.3	82.5±24.7	38.3±18.7	>4th D

P* value <0.05 is significant; D - Donation

In the present study, gender wise intergroup comparison was also done. Table 13 shows result of various studies depending upon the increasing number of donations made and also at which particular donation values became significant. These observations give us insight about the minimum number of donations that could be safe for donation.

In the present study fall in serum ferritin values became significant when potential donors were compared with donors who had already donated for three times in the previous year and were donating for the fourth or more than four times. This indicates that donating three times yearly could be a safe limit for the donors preventing them from any ill effect of iron deficiency and ensuring donor safety. The present study is consistent with the studies done by Abdul Aziz *et al* [55], Mittal *et al*[1]and Badar *et al*[8]. Our results compare well with the study done by Badar *et al*[8]. Studies done by Mittal *et al*[1]and Abdul Aziz *et al* [55]found significant fall in iron stores even with single donation that again confirms serum ferritin as the most sensitive readily available method for detection of mild iron depletion. Observation of the present study are nearly similar to the studies conducted by Mackintosh and Jacob[59] and Linpisarn *et al*[60]

Table 14: Mean serum ferritin levels (ng/ml) in different studies based on the number of donations in female donors:

Studies	1st time	2nd time	3rd time	≥4th time
Present study	33.4±33.3	20.0±18.0	5.2±NA	20.9±25.7
Abdul Aziz *et al* [55]	52.5±34.7	44.3±39.2	11.4±1.6	25±18.1
Martina NA[61]	71.3±35.0	56.3±38.2	76.4±44.3	NA
Mittal *et al*[1]	24.1±30.3	20.4±17.7	17.2±9.5	2.0 ± 2.8

In the present study, we observed that the mean serum ferritin values in female donors did not fall much with repeated donation and the difference was not significant. The present study was very close to the study conducted by Mittal *et al*[1] and Martina NA[61] but the findings of Morse *et al*[62] was somewhat different. Their study observed significant fall in serum ferritin level with increasing number of donation in female donors as well.

Also the mean serum ferritin values in first time female donors were lower compared to male donors. These results are consistent with the observations made by Mittal *et al*[1], Alvarez-Ossorio *et al*[63], Finch *et al*[64], Simon *et al*[44] and Abdul Aziz *et al* [55]except for the study done by Martina NA[61]. This difference could be because Martina NA[61]included educated donors who took appropriate care of their health. Hence, this comparison again tells about the importance of education and awareness in maximizing the female donor pool and safety.

According to these authors, female donors have a basal iron requirement of 1.3mg/day and iron stores were approximately 30 per cent lower than in men who gave one unit of blood yearly. However, both the groups had similar iron requirements and this difference was probably because of the higher iron intake in

male donors due to their higher caloric intake and monthly loss of blood in female donors. This may explain low initial serum ferritin values in female donors.

The present study also showed higher number of iron deficient female donors compared to male donors. This was seen both in the first time and regular blood donors.

Other hematological parameters:

Hematological parameters such as hemoglobin, hematocrit, MCH, MCV and RDW were also included in the present study. Observation of the results of the present study clearly indicates a greater fall of SF in **group B** of first time donors than **group A** (p=0.01) who donated more than once while hemoglobin, hematocrit, MCH, MCV, RDW; hematological parameters which are normally used for identification of anemia, have similar mean values within normal range. Such observations suggest that the total iron store as portrayed by SF level, even when it was at the lower level could not be identified by the routine hemoglobin values. Further, a greater degree of fall in SF level, as observed in group B (one donor who was donating for >4th time had shown SF value as 2.1ng/ml, but the haemoglobin level was 15gm/dl) could not be identified by the well-recognized cut off value of normal range of hemoglobin. Several researchers have similar observations confirming the fact that hemoglobin level estimation alone does not predict iron deficiency in a blood donor at the time of blood donation.

Below are the studies which had included other hematological parameters. These studies as well as the present study reflect the importance of serum ferritin.

Table 15: Hemoglobin values (mean ± SD) in gm /dl in various studies and p value:

Study	Gender	1st time donors	Repeat donors	p value
Present study	Male	14.5±1.4	14.8±2.0	>0.05
	Female	13.6±1.3	13.2±0.8	>0.05
Abdul Aziz *et al* [55]	Male	15.8±1.0	15.4±1.2	>0.05
	Female	13.6±0.9	13.6±0.6	>0.05
Jacob *et al* [52]	Male	16.1±1.1	16.2±1.0	>0.05
Norashikin *et al*[53]	Male	14.9±1.1	15.5 ±1.48	**<0.05***
Lim *et al*[56]	Male & Female	14.2±1.7	14.2±1.4	>0.05
Nadarajan *et al*[54]	Male & Female	13.8±1.6	13.9±1.4	>0.05
Martina NA[61]	Female	13.9±0.4	13.0±0.9	>0.05
Garg *et al*[2]	Male	14.2±0.9	14.1±0.9	>0.05
Gaff *et al*[15]	Male	15.2±1.2	15.4± 1.2	>0.05
Boulahriss *et al* [16]	Female	13.0±0.8	10.0±1.0	**<0.05**
Milman *et al*[57]	Female	13.7±0.1	13.9±1.1	>0.05

P * value <0.05 is significant

The fall in hemoglobin mean values with the number of donation was not significant and this observation was consistent with the work of Abdul Aziz *et al*[55], Jacob *et al*[52], Lim *et al*[56], Nadarajan *et al*[54], Martina NA[61], Garg *et al*[2], Gaff *et al*[15]and Milman *et al*[57]. Norashikin *et al*[53] observed significant fall in haemoglobin values after 5th donation and Boulahriss *et al*[16] documented this significance with a group which included donors who had donated at least 10 donations as compared to first time donors.

Table 16: Hematocrit values (mean ± SD) in % in various studies and p value:

Study	Gender	1st time donors	Repeat donors	p value
Present study	Male	42.9±3.9	44.5±6.1	>0.05
	Female	40.8±4.2	39.1±2.6	>0.05
Jacob *et al* [52]	Male	46.4±3.1	45.1±2.5	**<0.05**
Nadarajan *et al*[54]	Male & Female	43.0±5.0	44.0±4.0	>0.05
Garg *et al*[2]	Male	42.0±2.8	42.0±3.5	>0.05
Gaff *et al*[15]	Male	46.2± 3.4	45.4±3.3	**<0.05**
Lim *et al*[56]	Male & Female	42.5±5.2	42.8±4.2	>0.05

P * value <0.05 is significant

The findings of the present study for haematocrit values were consistent with the findings of Nadarajan *et al*[54], Garg *et al*[2]and Lim *et al*[56.] All showed that haematocrit values do not fall significantly with the number of donations. Jacob *et al* [52] and Gaff *et al*[15] had different observations. They found that hematocrit values fall significantly with increasing number of donations.

Table 17: MCV values (mean ± SD) in fl in various studies and p value:

Study	Gender	1st time donors	Repeat donors	p value
Present study	Male	86.2±11.0	87.5±6.9	>0.05
	Female	85.6±6.8	84.1±6.3	>0.05
Jacob *et al* [52]	Male	87.7±7.2	86.6±5.5	>0.05
Nadarajan *et al*[54]	Male & Female	86.2±6.7	85.2±6.2	>0.05
Garg *et al*[2]	Male	92.9±8.4	89.9±7.9	**0.05***
Lim *et al*[56]	Male & Female	88.3±4.1	85.9±7.0	>0.05
Abdullah *et al*[6]	Male	73.2±NA	73.2±NA	>0.05
Gaff *et al*[15]	Male	87.5±4.2	86.3±5.7	**<0.05***

P * value <0.05 is significant

MCV values in first time and multiple time donors were also studied. These values reflected similar observations as by Jacob *et al*[52], Nadarajan *et al*[54], Lim *et al*[56] and Abdullah *et al*[6]. While Garg *et al*[2]and Gaff *et al*[15]found significant fall in MCV values.

Table 18: MCH values (mean ± SD) in pg in various studies and p value:

Study	Gender	1st time donors	Repeat donors	p value
Present study	Male	29.5±2.7	29.2±3.0	>0.05
	Female	28.8±2.7	28.3±1.9	>0.05
Jacob *et al* [52]	Male	30.3±1.9	31.1±1.9	>0.05
Nadarajan *et al*[54]	Male & Female	27.2±2.5	27.0±2.3	>0.05
Garg *et al*(2)	Male	31.3±2.9	30.5±3.5	>0.05
Lim *et al*[56]	Male & Female	29.5±1.4	28.7±2.6	>0.05
Abdullah *et al*[6]	Male	27.4±NA	26.7±NA	>0.05
Gaff *et al*[15]	Male	28.8±2.1	29.4±2.3	>0.05

p value <0.05 is significant

All the authors found that MCH values do not fall significantly with increase in the number of donations which was consistent with the results of present study.

Table 19: MCHC values (mean ± SD) in % in various studies and p value:

Study	Gender	1st time donors	Repeat donors	p value
Present study	Male	33.8±1.3	33.2±1.4	**<0.05***
	Female	33.3±1.1	33.7±0.8	>0.05
Jacob *et al* [52]	Male	34.7±2.0	35.9±1.8	>0.05
Nadarajan *et al*[54]	Male & Female	31.5±1.2	31.4± 3.0	>0.05
Garg *et al*[2]	Male	33.8±1.6	33.9±2.1	>0.05
Lim *et al*[56]	Male & Female	33.4±0.5	33.4±0.9	>0.05

P * value <0.05 is significant

However, in the present study observation of significant fall in MCHC level with donation did not correlated with the studies done by Jacob *et al* [52], Nadarajan *et al*[54], Garg *et al*[2]and Lim *et al*[56] But this observation was within normal range of MCHC values.

Table 20: RDW values (mean ± SD) in % in various studies and p value:

Study	Gender	1st time donors	Repeat donors	p value
Present study	Male	14.0±4.1	13.8±1.4	>0.05
	Female	14.1±3.3	13.8±2.0	>0.05
Nadarajan *et al*[54]	Male & Female	15.1±1.4	15.5±1.8	>0.05
Garg *et al*[2]	Male	13.7±1.1	13.9±1.4	>0.05
Lim *et al*[56]	Male & Female	13.1±0.6	13.5±1.1	>0.05

p value <0.05 is significant

Nadarajan *et al*[54], Garg *et al*[2]and Lim *et al*[56] observed results which were consistent with the present study that is, no significant difference was observe in RDW values with increasing number of donation.

CONCLUSION

The present study indicates that SF level estimation in screened blood donors is essential to understand iron store status. Therefore, it may be introduced as a screening test before donating blood especially in repeat blood donors even though their hemoglobin level is normal.

In our country, the iron stores in females are low especially in the reproductive age group. Hence, serum ferritin evaluation needs to be included in the testing of first time female donors for donor safety. Furthermore, Iron supplementation should be routinely advised to repeat blood donors.

It is time to shift attention back to donor health, which is as important as growing need for a safe blood supply.

SUMMARY

- Present study was conducted as a cross-sectional study from 1st November 2013 to 30th June 2015.
- Total 235 donors were registered.
- After blood donation of 350/450 ml of whole blood, additional samples were collected in plain and EDTA vacutainers.
- The samples were processed within 4-6 hours of collection.
- EDTA blood was tested using the 5 part differentiated automated Hematoanalyzer and routine haematological parameters like haemoglobin, haematocrit, MCV, MCH, MCHC, RDW of the sample were measured.
- Serum ferritin estimation as a measure of iron status was done using ELISA technique.
- Donors were divided into groups according to the increasing number of donation made.
- Inter-group and inter-gender comparison of mean values of serum ferritin.
- Inter-group comparison of other haematological parameters - Haemoglobin, haematocrit, MCV, MCH, MCHC and RDW was done.
- Haematological parameters including mean haemoglobin values were within normal range and did not show any significant fall.
- Serum ferritin values decreased significantly with increase in the number of donations indicating fall in iron stores.
- Hence, SF level estimation may be introduced as a screening test in regular blood donors before blood donation even though their haemoglobin level is normal.
- Iron supplementation should be routinely advised to regular blood donors.

LIMITATIONS OF THE STUDY

As it is a requirement for the partial fulfilment of the MD pathology course were the candidate has to complete the study in a limited span of time, the sample size is relatively smaller, which may not give the representative findings of the population.

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ANNEXURE-I

ETHICAL CLEARANCE

B.L.D.E.UNIVERSITY'S
SHRI.B.M.PATIL MEDICAL COLLEGE, BIJAPUR-586 103
INSTITUTIONAL ETHICAL COMMITTEE

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Ethical Committee of this college met on 13-11-2013 at 3-30pm to scrutinize the Synopsis of Postgraduate Students of this college from Ethical Clearance point of view. After scrutiny the following original/corrected & revised version synopsis of the Thesis has been accorded Ethical Clearance.

Title "Utility of estimation of Serum ferritin as a screening test for blood donors - A cross-sectional study" — x — x —

Name of P.G. student Dr. Ila Singh
Department of Pathology

Name of Guide/Co-investigator Dr B. R. Yelikar
Prof & HOD. of Pathology

DR.TEJASWINI. VALLABHA
CHAIRMAN
INSTITUTIONAL ETHICAL COMMITTEE
BLDEU'S, SHRI.B.M.PATIL
MEDICAL COLLEGE, BIJAPUR.

Following documents were placed before E.C. for Scrutinization
1) Copy of Synopsis/Research project.
2) Copy of informed consent form
3) Any other relevant documents.

ANNEXURE-II

RESEARCH INFORMED CONSENT FORM

TITLE OF THE PROJECT	**:**	UTILITY OF ESTIMATION OF SERUM FERRITIN AS A SCREENING TEST FOR BLOOD DONORS
PRINCIPAL INVESTIGATOR	**:**	Dr. ILA SINGH P.G. DEPARTMENT OF PATHOLOGY
P.G.GUIDE	**:**	DR.BALASAHEB R.YELIKAR PROFESSOR AND HEAD DEPARTMENT OF PATHOLOGY

PURPOSE OF RESEARCH:

I have been informed that this study is done to assess the utility of estimation of serum ferritin as a screening test for blood donors.

PROCEDURE:

I understand that after blood donation of whole blood, additional samples will be collected in plain and EDTA vacutainers and given for complete blood count analysis.

RISK AND DISCOMFORTS:

I understand that, there is no risk involved in the procedures performed.

BENEFITS:

I understand that my participation in the study will help to know that serum ferritin level estimation in screened blood donors is essential to understand iron store status.

CONFIDENTIALITY:

I understand that the medical information produced by the study will become a part of hospital record and will be subjected to confidentiality and privacy regulations of the hospital. If the data is used for publications the identity of patient will not be revealed

REQEUST FOR MORE INFORMATION:

I understand that I may ask more information about the study at any time.

REFUSAL FOR WITHDRAWAL OF PARTICIPATION:

I understand that my participation is voluntary and that I may refuse to participate or may withdraw from the study at any time.

INJURY STATEMENT:

I understand that in the unlikely event of injury to me during the study I will get medical treatment but no further compensations.

I have read and fully understood this consent form. Therefore I agree to participate in the present study.

_____ _____

Participant / Guardian Date:

_____ _____

Signature of Witness Date:

I have explained the patient the purpose of the study, the procedure required and possible risk and benefit to the best of my ability in the vernacular language.

_____ _____

Investigator / P.G. Date:

_____ _____

Witness to Signature Date

ANNEXURE-III

BLOOD DONOR QUESTIONNAIRE AND CONSENT FORM/VOLUNTARY DONOR

SELECTION/REJECTION FORM

LICENCE NO.:KTK/28C-56/97 BLOOD UNIT NO.: BLOOD GROUP AND Rh

TYPE:

DATE OF COLLECTION: EXPIRY DATE:

CONFIDENTIAL

Name: Age: Sex: D.O.B.:

Contact No.:

Occupation: Address for communication:

a) Would you like us to call on your mobile:

YES/NO

b) Have you donated previously:

YES/NO

if yes then on how many occasions: When last donated:

c) Your blood group: Time of last meal:

Did you have discomfort during donation:

1. Do you feel well today?

YES/NO

2. Did you have something to eat in the last 4hrs?

YES/NO

3. Did you sleep well last night?

   YES/NO

4. Have you any reason to believe that you may be infected by either hepatitis, malaria, HIV/AIDS, and/or veneral disease?

   YES/NO

5. in the last 6 months have you any history of following:

   Unexplained weight loss: Repeated diarrhea: Swollen glands:

6. In the last 6 months have you had any :

   Tattooing: Ear Peircing: Dental extraction:

7. Do you suffer from or have suffered from any of the following diseases?

Heart disease: Lung disease: Kidney disease: STD:

diabetes: Tuberculosis: Jaundice: Malaria:

Hepatitis B/C: Cancer: Epilepsy: Fainting

Spells:

Allergic Disease: Abnormal Bleeding Tendency: Typhoid:

Are you taking or have taken any of these in the past 72 hrs:

Antibiotics: Aspirin: Alcohol: Steroids: Vaccination:

Dog Bite:

8. Is there any history of surgery or blood transfusion in the past 6 months

Major surgery: Minor surgery: Blood

transfusion:

9. Women Donors:

a) Are you pregnant?

   YES/NO

b) Have you had an abortion in the last 3months?

YES/NO

c) Do you have a child less than 1yr old?

YES/NO

d) Are you having your periods today?

YES/NO

10. would you like to be informed about any abnormal test result at the address furnished by you.

YES/NO

11. Have you read and understood all the information presented and answered all the questions truthfully, as any incorrect statement or concealment may affect your health ao may harm the recipient.

YES/NO

I UNDERSTAND

A. Blood donation is a totally voluntary act and no inducement or remuneration has been offered.

B. Donation of blood /component is a medical procedure and that by donating voluntarly,I accept the risk associated with the procedure.

C. My blood will be tested for Hepatitis B,Hepatitis C,Malarial parasite, HIV/AIDS and veneral diseases in addition to any other screening tests required to ensure blood safety.

I prohibit any information provided by me or about my donation to be disclosed to any individual or government agency without my prior permission.

DATE: TIME: DONOR SIGNATURE:

General physical examination:

Weight: Pulse: Hb: Temperature: BP:

Accept: Defer: Reason:

Signature of Medical officer

ANNEXURE IV

PROFORMA FOR STUDY

Demographic Details:

1. Name: 2. Age: 3. Sex: M/F 4. OPD / IPD no.:

5. Present history:

6. Past history :

7. History of intake of drugs:

8. General physical examination**:**

Pallor : Icterus :

Built : Nourishment :

9. Vitals:-

PULSE : BP :

RR :

Temp : Weight :

10. Investigations:

11. Hematological examination

Hb: Hematocrit : MCV: MCHC: MCHC: RDW: Serum ferritin:

Parameters studied and their normal reference values.[65]

Parameters	Gender	Reference values
Hemoglobin (g/dl)	Males Females	13.5- 18 12.5-16
Hematocrit (%)	Males Females	42- 52 37- 47
MCV (fl)		78- 100
MCH (pg)		27- 31
MCHC(%)		33- 37
RDW(%)		11.6- 14
Serum Ferritin (ng/ml)	Male	30- 300
	Female	10- 200

KEY TO MASTER CHART

Hb	Hemoglobin
PCV	Packed Cell Volume
MCV	Mean Corpuscular Volume
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
RDW	Red cell Distribution Width
SF	Serum Ferritin
M	Male
F	Female
nd	Number of donations as applicable and mentioned in the present study

MASTER CHART

	sex	no. of donation	Hb(g/dl)	PCV(%)	MCV(fl)	MCH(pg)	MCHC	RDW	SF(ng/dl)	Hb(g/dl)	nd
1.	M	1.00	12.50	37.30	86.50	28.30	33.50	13.00	25.40	12.50	1.00
2.	F	1.00	12.50	39.00	91.30	28.10	32.10	16.40	171.20	12.50	1.00
3.	M	1.00	13.60	42.10	89.40	28.90	32.30	14.90	60.70	13.60	1.00
4.	M	1.00	12.80	40.00	81.80	26.20	32.00	13.80	23.00	12.80	1.00
5.	M	1.00	12.70	37.50	84.10	28.50	33.90	12.00	96.10	12.70	1.00
6.	M	1.00	14.20	41.50	88.30	30.20	34.20	13.30	64.90	14.20	1.00
7.	M	1.00	14.60	42.00	89.90	31.30	34.80	12.10	23.60	14.60	1.00
8.	M	1.00	16.70	48.50	93.60	32.20	34.40	14.70	104.40	16.70	1.00
9.	M	1.00	14.30	40.70	86.40	30.40	35.10	13.10	26.30	14.30	1.00
10.	M	1.00	17.00	51.50	91.60	30.20	33.00	12.60	76.80	17.00	1.00
11.	M	1.00	12.50	37.40	91.70	29.40	33.40	12.50	73.80	12.50	1.00
12.	M	1.00	14.50	43.80	83.80	27.70	33.00	12.80	79.10	14.50	1.00
13.	M	1.00	13.50	39.70	76.90	26.20	34.00	12.40	110.20	13.50	1.00
14.	M	1.00	12.50	38.00	85.80	28.00	32.90	12.80	83.00	12.50	1.00
15.	M	1.00	12.80	39.00	85.00	28.10	33.10	13.40	285.30	12.80	1.00
16.	M	1.00	13.00	38.00	82.30	28.10	34.20	15.00	25.40	13.00	1.00
17.	M	1.00	13.20	39.20	104.30	35.10	33.70	13.40	67.10	13.20	1.00
18.	M	1.00	12.50	37.10	86.10	27.80	33.70	12.10	73.10	12.50	1.00
19.	M	1.00	15.60	49.30	84.90	26.90	31.60	43.10	451.10	15.60	1.00
20.	M	1.00	13.80	38.10	82.80	30.00	36.20	11.90	23.90	13.80	1.00
21.	M	1.00	14.00	41.10	82.20	28.00	34.10	12.00	45.90	14.00	1.00
22.	M	1.00	12.50	38.10	81.10	25.70	32.80	12.80	76.70	12.50	1.00
23.	F	1.00	12.20	35.40	77.60	26.80	34.50	14.10	20.90	12.20	1.00
24.	M	1.00	18.80	56.00	101.30	34.00	33.60	13.30	298.20	18.80	1.00
25.	F	1.00	13.30	40.90	89.10	29.00	32.50	13.20	71.10	13.30	1.00
26.	F	1.00	12.30	34.90	79.50	28.00	35.20	13.10	149.00	12.30	1.00

27.	F	1.00	12.60	38.10	90.90	30.10	33.10	12.90	54.00	12.60	1.00
28.	F	1.00	15.30	45.20	85.90	29.10	33.80	14.00	18.80	15.30	1.00
29.	M	1.00	15.20	45.10	85.70	28.90	33.70	13.90	14.40	15.20	1.00
30.	M	1.00	13.80	40.10	86.20	29.70	34.40	13.50	225.10	13.80	1.00
31.	F	1.00	12.50	37.70	79.00	26.20	33.20	14.50	54.20	12.50	1.00
32.	M	1.00	14.70	42.90	89.60	30.70	34.30	12.40	19.60	14.70	1.00
33.	M	1.00	16.40	46.30	85.40	30.30	35.40	12.10	71.90	16.40	1.00
34.	F	1.00	13.60	39.00	85.30	29.80	34.90	12.50	5.60	13.60	1.00
35.	M	1.00	14.60	41.80	85.10	29.70	34.90	12.80	20.50	14.60	1.00
36.	M	1.00	16.80	45.70	81.30	29.90	36.80	12.30	39.00	16.80	1.00
37.	F	1.00	12.90	38.50	100.80	33.80	33.50	14.40	1.50	12.90	1.00
38.	M	1.00	15.30	43.20	79.90	28.30	35.40	13.00	2.50	15.30	1.00
39.	M	1.00	15.50	47.40	83.60	27.30	32.70	12.10	21.70	15.50	1.00
40.	M	1.00	12.50	38.40	93.20	30.30	32.60	13.50	23.20	12.50	1.00
41.	M	1.00	13.30	41.00	84.90	27.50	32.40	12.90	18.40	13.30	1.00
42.	F	1.00	12.60	39.10	75.60	24.40	32.20	14.80	45.30	12.60	1.00
43.	F	1.00	13.70	40.70	87.30	29.40	33.70	14.00	8.20	13.70	1.00
44.	F	1.00	14.50	43.00	85.70	28.90	33.70	12.50	10.10	14.50	1.00
45.	F	1.00	13.20	39.10	88.10	29.70	33.80	12.80	12.60	13.20	1.00
46.	F	1.00	13.60	39.70	89.00	30.50	34.30	15.20	36.30	13.60	1.00
47.	F	1.00	14.00	41.60	85.80	28.90	33.70	12.50	4.60	14.00	1.00
48.	F	1.00	12.50	40.40	77.00	23.80	30.90	15.80	36.50	12.50	1.00
49.	F	1.00	11.00	44.60	99.10	31.10	31.40	13.10	54.80	11.00	1.00
50.	F	1.00	14.10	42.90	85.50	28.10	32.90	13.50	25.30	14.10	1.00
51.	F	1.00	14.00	43.90	81.40	26.20	32.10	14.00	25.30	14.00	1.00
52.	F	1.00	14.00	41.80	92.30	30.90	33.50	14.20	23.70	14.00	1.00
53.	F	1.00	13.90	41.20	99.30	33.50	33.70	13.70	91.10	13.90	1.00
54.	F	1.00	13.40	42.90	85.50	26.70	31.20	13.60	7.30	13.40	1.00
55.	F	1.00	12.60	38.90	83.80	27.20	32.40	13.10	14.30	12.60	1.00
56.	F	1.00	14.20	43.90	81.80	26.40	32.30	13.30	30.50	14.20	1.00
57.	M	1.00	14.10	43.60	86.30	27.90	32.30	13.20	88.00	14.10	1.00

58.	M	1.00	15.70	45.30	84.70	29.30	34.70	13.20	30.30	15.70	1.00
59.	M	1.00	14.10	42.90	98.20	32.30	32.90	14.00	38.00	14.10	1.00
60.	M	1.00	14.50	43.80	83.80	27.70	33.00	12.80	13.80	14.50	1.00
61.	M	1.00	16.10	49.30	89.30	29.00	32.50	13.20	20.40	16.10	1.00
62.	M	1.00	13.70	42.60	85.90	27.60	32.20	17.70	15.50	13.70	1.00
63.	M	1.00	14.70	46.60	80.60	25.40	31.50	13.30	15.00	14.70	1.00
64.	M	1.00	13.60	44.20	82.00	25.20	30.80	15.50	11.00	13.60	1.00
65.	M	1.00	17.30	49.60	85.20	29.70	34.90	14.10	20.10	17.30	1.00
66.	M	1.00	15.30	44.20	86.20	29.80	34.60	13.00	31.70	15.30	1.00
67.	M	1.00	15.60	46.60	96.30	32.20	33.50	14.00	15.50	15.60	1.00
68.	M	1.00	15.60	43.10	86.70	31.40	36.20	11.80	15.20	15.60	1.00
69.	M	1.00	13.70	41.80	9.30	30.60	32.80	14.70	23.70	13.70	1.00
70.	M	1.00	14.60	43.10	88.70	30.00	33.90	12.60	14.80	14.60	1.00
71.	M	1.00	14.20	43.60	83.50	27.20	32.60	13.90	22.50	14.20	1.00
72.	M	1.00	16.20	46.90	92.50	32.00	34.50	14.60	72.60	16.20	1.00
73.	M	1.00	13.40	44.20	63.40	19.20	30.30	18.20	110.00	13.40	1.00
74.	M	1.00	14.70	43.20	93.30	31.70	34.00	13.80	284.20	14.70	1.00
75.	M	1.00	14.90	45.80	81.30	26.50	32.50	12.50	23.70	14.90	1.00
76.	M	1.00	15.10	41.50	90.20	32.80	36.40	15.40	205.20	15.10	1.00
77.	M	1.00	15.80	42.70	80.00	29.60	37.00	13.30	71.90	15.80	1.00
78.	M	1.00	13.30	39.90	100.50	33.50	33.30	17.00	56.20	13.30	1.00
79.	M	1.00	16.60	50.30	87.80	29.00	33.00	12.10	150.70	16.60	1.00
80.	F	1.00	18.00	54.00	93.30	31.10	33.30	13.20	26.30	18.00	1.00
81.	M	1.00	15.00	43.20	84.00	29.20	34.70	13.50	206.90	15.00	1.00
82.	M	1.00	15.00	44.80	78.20	26.20	33.50	14.30	82.50	15.00	1.00
83.	F	1.00	13.80	40.00	84.60	29.20	34.50	14.00	60.10	13.80	1.00
84.	F	1.00	12.50	37.40	82.60	27.40	33.20	13.00	52.10	12.50	1.00
85.	F	1.00	14.00	44.60	82.00	31.40	31.40	13.20	24.20	14.00	1.00
86.	F	1.00	16.90	50.30	85.40	33.60	33.60	12.90	14.90	16.90	1.00
87.	F	1.00	14.90	43.40	88.00	34.30	34.30	13.40	46.00	14.90	1.00
88.	F	1.00	14.50	44.90	88.70	32.30	32.30	13.10	56.20	14.50	1.00

89.	F	1.00	13.50	39.70	76.90	26.20	34.00	17.00	47.50	13.50	1.00
90.	F	1.00	13.40	42.10	74.80	23.80	31.80	13.20	19.50	13.40	1.00
91.	F	1.00	13.10	39.90	79.00	25.90	32.80	12.80	17.50	13.10	1.00
92.	F	1.00	18.10	55.30	93.40	30.60	32.70	12.70	15.20	18.10	1.00
93.	F	1.00	13.10	38.10	80.20	27.60	34.40	14.50	20.30	13.10	1.00
94.	F	1.00	15.80	49.00	83.10	26.80	32.20	12.70	21.10	15.80	1.00
95.	M	1.00	14.00	42.30	93.80	31.00	33.10	12.70	6.60	14.00	1.00
96.	M	1.00	13.20	38.80	84.00	28.60	34.00	12.00	11.40	13.20	1.00
97.	M	1.00	18.00	51.50	98.50	34.40	35.00	12.00	73.20	18.00	1.00
98.	M	1.00	14.50	41.70	84.20	29.30	34.80	11.70	82.10	14.50	1.00
99.	M	1.00	14.30	42.30	84.90	28.70	33.80	13.70	62.10	14.30	1.00
100.	F	1.00	14.00	40.10	81.30	28.40	34.90	11.90	15.20	14.00	1.00
101.	M	1.00	12.80	38.90	84.40	27.80	32.90	17.30	73.30	12.80	1.00
102.	F	1.00	12.60	37.20	92.10	31.20	33.90	12.60	14.00	12.60	1.00
103.	M	1.00	13.50	39.10	83.70	28.90	34.50	12.30	244.80	13.50	1.00
104.	M	1.00	14.50	42.10	85.60	29.50	34.40	12.60	105.50	14.50	1.00
105.	M	1.00	15.60	47.50	92.80	30.50	32.80	14.10	82.40	15.60	1.00
106.	F	1.00	13.60	41.50	91.20	29.90	32.80	14.00	20.00	13.60	1.00
107.	F	1.00	12.70	37.40	81.70	27.70	34.00	13.20	8.90	12.70	1.00
108.	M	1.00	13.00	37.10	85.30	29.90	35.00	31.10	411.50	13.00	1.00
109.	M	1.00	14.80	41.20	112.30	40.30	35.90	17.00	140.00	14.80	1.00
110.	M	1.00	15.50	43.30	92.10	33.00	35.80	12.80	182.00	15.50	1.00
111.	M	1.00	15.00	43.40	90.80	31.40	34.60	12.20	138.00	15.00	1.00
112.	M	1.00	15.40	43.30	96.70	34.40	35.60	14.60	262.00	15.40	1.00
113.	M	1.00	14.40	41.20	89.00	31.10	35.00	13.00	19.00	14.40	1.00
114.	F	1.00	12.20	34.00	80.00	28.70	35.90	13.20	9.30	12.20	1.00
115.	M	1.00	16.30	46.90	76.90	26.70	34.80	13.40	4.90	16.30	1.00
116.	M	1.00	16.10	46.50	87.70	30.40	34.60	11.90	20.10	16.10	1.00
117.	F	1.00	13.00	40.60	80.20	25.70	32.00	14.60	25.20	13.00	1.00
118.	F	1.00	13.40	39.40	88.30	30.00	34.00	13.40	17.10	13.40	1.00
119.	M	1.00	14.10	43.10	84.50	27.60	32.70	12.00	21.10	14.10	1.00

120.	F	1.00	12.50	38.60	63.30	20.50	32.40	21.20	10.30	12.50	1.00
121.	F	1.00	13.60	41.50	91.20	29.90	32.80	14.00	77.00	13.60	1.00
122.	M	1.00	14.20	41.80	82.00	27.80	34.00	13.20	45.30	14.20	1.00
123.	M	1.00	12.50	38.10	87.80	28.10	32.80	13.30	8.90	12.50	1.00
124.	F	1.00	12.50	35.90	88.00	30.40	34.50	12.90	48.20	12.50	1.00
125.	M	1.00	13.00	39.40	88.30	29.10	33.00	13.90	92.20	13.00	1.00
126.	M	1.00	15.10	46.30	89.60	29.20	32.60	13.20	16.20	15.10	1.00
127.	F	1.00	13.10	38.90	88.20	29.70	33.70	12.30	11.50	13.10	1.00
128.	F	1.00	13.50	38.90	93.70	32.50	34.70	13.90	23.20	13.50	1.00
129.	F	1.00	12.70	36.20	85.40	28.20	32.50	35.00	2.20	12.70	1.00
130.	F	1.00	12.80	37.70	80.60	27.40	34.00	13.20	5.60	12.80	1.00
131.	F	1.00	12.60	37.30	91.60	31.00	33.80	11.80	76.20	12.60	1.00
132.	M	1.00	13.50	37.90	91.80	32.70	35.60	12.50	28.50	13.50	1.00
133.	F	1.00	13.40	38.60	88.90	30.90	34.70	14.30	14.40	13.40	1.00
134.	M	2.00	16.60	51.30	88.60	28.70	32.40	15.80	172.10	16.60	2.00
135.	M	2.00	12.50	36.00	88.00	30.10	34.70	13.70	57.40	12.50	2.00
136.	M	2.00	14.00	42.30	85.80	28.40	33.10	13.60	4.05	14.00	2.00
137.	M	2.00	17.30	52.40	87.90	29.00	33.00	12.70	39.94	17.30	2.00
138.	M	2.00	20.90	66.60	89.30	28.00	31.40	13.40	24.82	20.90	2.00
139.	M	2.00	17.60	56.10	93.20	29.20	31.40	13.90	14.28	17.60	2.00
140.	M	2.00	17.50	55.00	88.30	28.10	31.80	14.60	49.94	17.50	2.00
141.	M	2.00	12.50	39.60	90.40	28.10	31.60	15.50	29.20	12.50	2.00
142.	M	2.00	12.50	39.70	93.20	29.10	31.50	14.10	32.09	12.50	2.00
143.	M	2.00	15.20	45.30	82.40	27.60	33.60	13.10	53.57	15.20	2.00
144.	M	2.00	13.50	40.30	82.80	27.70	33.50	13.20	163.70	13.50	2.00
145.	M	2.00	16.00	46.30	88.40	30.50	34.60	14.80	69.50	16.00	2.00
146.	M	2.00	13.20	40.80	87.20	28.20	32.40	15.00	53.80	13.20	2.00
147.	M	2.00	16.40	48.30	83.60	28.40	34.00	13.20	6.00	16.40	2.00
148.	M	2.00	16.00	49.20	83.20	27.10	32.50	13.40	34.70	16.00	2.00
149.	M	2.00	13.00	39.40	99.00	32.70	33.00	14.60	50.70	13.00	2.00
150.	M	2.00	14.50	43.40	91.80	30.70	33.40	13.30	31.00	14.50	2.00

151.	M	2.00	15.40	45.10	85.40	29.20	34.10	12.70	36.20	15.40	2.00
152.	M	2.00	13.80	41.10	108.40	36.40	33.60	14.90	108.30	13.80	2.00
153.	M	2.00	12.50	36.20	88.90	29.50	34.50	13.50	82.90	12.50	2.00
154.	M	2.00	12.80	40.80	81.60	25.60	31.40	13.60	54.60	12.80	2.00
155.	M	2.00	12.90	41.30	85.20	26.60	31.20	13.90	12.30	12.90	2.00
156.	F	2.00	14.20	43.30	76.40	25.00	32.80	14.10	40.10	14.20	2.00
157.	M	2.00	13.50	42.80	80.40	26.80	31.20	14.30	41.00	13.50	2.00
158.	M	2.00	14.30	41.80	95.90	32.80	34.20	14.50	52.30	14.30	2.00
159.	M	2.00	14.80	44.60	97.40	32.30	33.20	15.30	34.10	14.80	2.00
160.	M	2.00	13.90	40.60	89.80	30.80	34.20	13.40	23.40	13.90	2.00
161.	M	2.00	14.60	43.80	81.60	27.20	33.30	11.50	6.50	14.60	2.00
162.	M	2.00	14.40	42.40	81.20	27.60	34.00	13.20	41.50	14.40	2.00
163.	M	2.00	15.30	45.70	90.30	30.20	33.50	14.40	21.80	15.30	2.00
164.	M	2.00	15.80	47.30	80.70	27.00	33.40	13.40	34.10	15.80	2.00
165.	M	2.00	16.50	51.80	84.80	27.00	31.90	11.90	108.30	16.50	2.00
166.	M	2.00	14.00	38.80	81.70	29.50	36.10	12.70	115.00	14.00	2.00
167.	M	2.00	16.80	45.40	93.40	34.60	37.00	11.90	52.30	16.80	2.00
168.	F	2.00	13.10	38.90	91.30	30.80	33.70	13.70	53.99	13.10	2.00
169.	F	2.00	12.50	36.80	77.00	25.90	33.70	13.20	6.34	12.50	2.00
170.	M	2.00	15.10	46.30	76.80	25.00	32.60	13.00	30.50	15.10	2.00
171.	M	2.00	15.20	48.60	81.40	31.30	31.50	13.20	43.11	15.20	2.00
172.	M	2.00	13.70	42.00	88.40	32.60	30.80	12.70	150.23	13.70	2.00
173.	M	2.00	17.70	51.80	87.20	29.80	34.20	12.10	20.33	17.70	2.00
174.	M	2.00	12.50	39.20	87.90	28.00	31.90	17.20	62.32	12.50	2.00
175.	F	2.00	14.70	44.30	88.10	29.20	33.20	13.00	20.40	14.70	2.00
176.	F	2.00	13.50	39.10	81.60	28.20	34.50	13.10	2.10	13.50	2.00
177.	M	2.00	14.60	40.80	104.60	37.40	35.80	12.80	95.45	14.60	2.00
178.	M	2.00	14.40	43.20	83.10	27.70	33.30	12.30	146.99	14.40	2.00
179.	M	2.00	12.70	37.30	92.60	31.50	34.00	12.50	14.25	12.70	2.00
180.	M	2.00	12.60	38.00	86.00	28.50	33.20	13.00	10.80	12.60	2.00
181.	F	2.00	14.00	40.30	82.40	28.60	34.70	13.00	15.80	14.00	2.00

182.	M	2.00	14.50	43.90	87.50	28.90	33.00	13.00	24.20	14.50	2.00
183.	M	2.00	15.70	46.80	87.00	29.20	33.50	14.60	31.50	15.70	2.00
184.	F	2.00	12.50	36.50	83.10	28.50	34.20	13.80	10.60	12.50	2.00
185.	F	2.00	12.50	36.50	83.10	28.50	34.20	13.80	10.60	12.50	2.00
186.	M	2.00	14.40	43.20	83.10	27.70	33.30	12.30	39.70	14.40	2.00
187.	M	2.00	12.50	37.10	92.30	31.10	33.70	14.20	59.00	12.50	2.00
188.	M	3.00	13.80	38.00	86.20	31.30	36.30	13.10	88.17	13.80	3.00
189.	M	3.00	17.50	54.00	93.30	30.20	32.40	14.50	10.84	17.50	3.00
190.	M	3.00	15.80	47.60	82.80	27.50	33.20	12.70	26.70	15.80	3.00
191.	M	3.00	14.20	40.80	85.70	29.80	34.80	11.70	2.30	14.20	3.00
192.	M	3.00	14.90	42.70	86.10	30.00	34.90	12.10	1.00	14.90	3.00
193.	F	3.00	12.80	37.10	82.60	28.50	34.50	11.90	5.20	12.80	3.00
194.	M	3.00	16.60	49.00	89.70	30.40	33.90	13.20	12.80	16.60	3.00
195.	M	3.00	12.80	39.40	88.70	28.80	32.50	13.30	35.50	12.80	3.00
196.	M	3.00	15.10	44.40	83.50	28.40	34.00	14.50	3.70	15.10	3.00
197.	M	3.00	14.70	44.40	95.30	31.50	33.10	14.00	1.20	14.70	3.00
198.	M	3.00	14.80	41.50	85.40	31.60	32.10	13.30	107.00	14.80	3.00
199.	M	3.00	17.10	49.30	95.00	32.90	34.70	12.70	43.00	17.10	3.00
200.	M	3.00	12.50	36.50	102.50	34.30	34.20	14.10	92.00	12.50	3.00
201.	M	3.00	16.00	47.80	85.70	28.90	33.70	13.20	92.00	16.00	3.00
202.	M	3.00	16.90	51.80	108.10	35.30	32.60	17.70	50.00	16.90	3.00
203.	M	3.00	13.60	41.10	87.40	28.90	33.10	13.50	13.60	13.60	3.00
204.	M	3.00	13.40	39.30	95.20	32.40	34.10	12.30	207.10	13.40	3.00
205.	M	5.00	12.50	39.60	82.80	25.90	31.60	12.70	68.97	12.50	4.00
206.	M	8.00	12.90	39.00	85.00	28.10	33.10	13.40	167.94	12.90	4.00
207.	M	4.00	12.50	35.20	90.70	30.90	35.50	12.00	55.45	12.50	4.00
208.	M	4.00	12.50	40.30	67.10	20.10	31.00	18.30	54.69	12.50	4.00
209.	M	9.00	17.20	54.50	89.50	28.20	31.60	13.80	23.31	17.20	4.00
210.	M	7.00	19.20	60.50	89.50	28.40	31.70	12.80	39.25	19.20	4.00
211.	M	6.00	13.40	42.40	81.90	25.90	31.60	12.80	8.50	13.40	4.00
212.	M	5.00	20.50	62.80	95.70	31.30	32.60	13.90	47.99	20.50	4.00

213.	M	9.00	17.80	51.60	79.40	27.40	34.50	13.00	85.90	17.80	4.00
214.	M	4.00	14.80	44.90	85.20	28.10	33.00	17.60	5.40	14.80	4.00
215.	M	7.00	13.60	40.10	79.20	26.90	33.90	14.00	9.40	13.60	4.00
216.	M	8.00	14.60	42.10	82.40	28.60	34.70	12.30	29.70	14.60	4.00
217.	M	9.00	15.10	42.70	92.60	32.80	35.40	16.40	92.00	15.10	4.00
218.	M	9.00	13.20	42.20	82.30	22.10	32.90	14.30	51.00	13.20	4.00
219.	M	4.00	12.80	40.10	92.40	29.50	31.90	15.00	68.00	12.80	4.00
220.	M	5.00	16.30	41.20	82.60	31.50	32.60	12.50	2.10	16.30	4.00
221.	M	6.00	14.90	46.70	78.00	24.90	31.90	13.70	13.00	14.90	4.00
222.	M	4.00	12.50	41.30	77.80	23.50	30.30	13.80	14.00	12.50	4.00
223.	M	7.00	13.90	41.20	96.00	32.40	33.70	13.60	10.00	13.90	4.00
224.	M	4.00	13.90	44.30	87.90	27.60	31.40	13.20	91.00	13.90	4.00
225.	M	8.00	14.50	41.60	86.30	30.10	34.90	15.90	58.00	14.50	4.00
226.	M	5.00	12.50	38.00	90.00	29.10	32.90	13.70	5.28	12.50	4.00
227.	M	7.00	18.00	55.40	86.40	29.50	34.10	13.80	61.22	18.00	4.00
228.	M	6.00	15.10	44.90	81.60	27.50	33.60	12.90	19.55	15.10	4.00
229.	M	4.00	14.80	46.20	82.60	32.00	31.20	13.90	103.43	14.80	4.00
230.	F	4.00	12.50	39.10	98.00	31.30	32.00	19.40	39.00	12.50	4.00
231.	M	4.00	12.70	41.80	69.90	20.10	28.70	18.30	2.70	12.70	4.00
232.	M	4.00	12.90	37.50	100.50	34.60	34.40	15.60	20.70	12.90	4.00
233.	F	5.00	12.70	38.00	81.20	27.10	33.40	12.50	2.70	12.70	4.00
234.	M	5.00	13.50	42.00	84.20	27.10	32.10	13.40	9.60	13.50	4.00
235.	M	4.00	20.40	58.30	86.80	30.40	35.00	13.50	21.30	20.40	4.00