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Assessment of iron status in regular blood donors in a tertiary care hospital in Southern India

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Abstract:

BACKGROUND: Regular blood donation depletes iron stores. The assertion is that the vulnerable donor population requires a predictive standard operative procedure for early detection of iron store depletion, preventing them from developing iron-deficiency anemia.

AIM: This study aims to study the potential effects of blood donation in the regular donor group using hematological and biochemical estimation of iron status parameters.

STUDY SETTINGS AND DESIGN: This was a prospective cross-sectional study on regular blood donors, defined as those who have donated at least 3 times, the last donation being within the last 12 months and continues to donate at least once a year, at a tertiary care teaching hospital in Southern India.

MATERIALS AND METHODS: The complete blood count (CBC) was performed on the Sysmex coulter, and the red cell indices were calculated. The ferritin and the soluble transferrin receptor (sTfR) assays were performed using Enzyme Immunoassays.

STATISTICAL ANALYSIS USED: The comparison of CBC, serum ferritin, and sTfR assay with donation frequency and time since the last donation was carried out using an independent student's *t*-test for two groups. The statistical analysis was performed using SPSS for Windows version 20.

RESULTS: A total of 323 regular blood donors (6 were females) were included in the study of which they were categorized into three, 211 donors with less than or equal to 10 donations, 84 those who had donated between 11 and 20 times and 28 who had donated more than 20 times. The red cell indices were reduced and different in the groups but not statistically significant except for mean corpuscular volume. About 15% of the study population had a transferrin level of <15 ng/ml. The Ferritin levels showed a statistically significant negative correlation with the number of donations, the correlation coefficient being -0.27 . Logarithmic ratios of sTfR/ferritin also correlated with a coefficient of 0.156 with the number of donations and were statistically significant.

CONCLUSION: Our study found that regular blood donors had low iron stores, as shown by ferritin levels and other iron indicators. Using the current guidelines (hemoglobin >12.5 g/dL) for donation, or the red cell indices alone do not reflect the donor's actual iron status.

Keywords:

Ferritin, iron status, regular blood donors, soluble transferrin receptor assay

Introduction

Iron deficiency is an increasingly recognized repercussion of blood donation. Donation

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of 450 ml of blood equates to approximately 200–250 mg of iron loss in the donor population.^[1] Progressive iron depletion leads to iron-deficient erythropoiesis (IDE), non-anemic iron deficiency, and ultimately iron-deficiency anemia.^[2]

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Even in the absence of anemia, iron deficiency imposes a strain on homeostasis, causing increased fatigue and impaired physical and cognitive performance in healthy individuals. It also has a significant impact on the physiology of premenopausal women and professional, regular blood donors.^[3] The prevalence of depleted iron stores in India resulting in anemic iron deficiency and non-anemic iron deficiency among the regular blood donor population is estimated to be at around 30%.^[1] Subsequent donations from such donors may result in undesired effects to the donor and may result in deferrals. Compared to non-deferred donors, those deferred for this reason are less likely to return for subsequent donations. Thus, donor iron deficiency can affect the inherent blood supply.^[4]

Regular blood donation depletes iron stores. The assertion is that the vulnerable donor population requires a predictive standard operative procedure (SOP) for early detection of iron store depletion, preventing them from developing iron-deficiency anemia. This study will provide an overview of the potential effects of blood donation in the regular donor group using hematological and biochemical estimation of iron status parameters. Such evaluation and estimation of iron store depletion on account of regular blood donation need to be evaluated and assessed, which will better serve the population that tends to the needs of the sick.

This study was designed to provide information on the hematological indices and iron status of voluntary regular blood donors at a single center. We studied serum ferritin levels and the sTfR assays to assess storage iron levels and functional iron deficiency, respectively. Performing a representative cross-sectional study to determine the status of iron stores in regular blood donors helps analyze the population at risk and develop potential anticipating methods to estimate the risk of iron deficiency in individual donors. Thereby, we can effectively avoid the morbidity of the donor population, encourage judicious, meticulous practices, and preserve the vulnerable donor cohort for sustainable resources.

This study aimed to assess the hematological and biochemical variations with respect to the iron status in regular blood donors in a Tertiary Care Hospital in South India with an objective to predict the adverse consequences of blood donations and identify the at-risk population, which helps serve the population that tends to the needs of the sick in a better and safe way.

Materials and Methods

Study setting and population

This was a prospective cross-sectional analytical study on regular blood donors conducted in the Transfusion

medicine department of 2100 bedded multi-specialty tertiary care teaching hospital in South-Eastern India. Regular blood donors who report to the blood center from first January 2019 to April 2020 were selected. All the donors selected were active donors, according to the records. The medical examination was normal and fulfilled the hemoglobin (Hb) criteria for donation (≥ 12.5 g/dL) as per our department SOP using the copper sulfate specific gravity method.

Sampling and sample size

The sample size was calculated using the statistical formula for approximating a single proportion. The expected prevalence of iron deficiency among regular blood donors was taken as 30% based on Datta *et al.*^[1] A sample size of 323 was estimated at a 5% level of significance and 5% absolute precision. A single group of 323 regular blood donors was recruited for the study by stratified random sampling. Stratification was based on the frequency of blood donations carried out earlier. A regular blood donor was defined as a donor who has donated at least 3 times, the last donation being within the last 12 months and continues to donate at least once a year.^[5] Donors who tested seropositive to transfusion transmissible infections or those on any form of iron supplementation were excluded from the study.

Study procedure

Post donation, 5 ml of the venous blood sample that is collected for routine blood grouping and serological testing for the donor was utilized for testing the necessary parameters. 2 ml of venous blood was transferred to EDTA Tube, which was used for estimation of hematological parameters such as Hb, red cell count, mean corpuscular volume (MCV), mean corpuscular Hb, mean corpuscular Hb concentration, and red cell distribution width. Red cell indices (Hb, Hct, MCV, mean corpuscular hemoglobin [MCH], MCHC, red blood cell distribution width [RDW]) were measured by using Sysmex (XT2000i) (Coulter, Japan). Combined cell index (CCI) was calculated by the formula: $CCI = RDW \times 10^4 \times MCV^{-1} \times MCH^{-1}$.^[6] Peripheral smear was stained with Leishman stain and was studied for red cell morphology. To avoid variations, all these smears were read by a single investigator, and opinion was sought from the other investigators to arrive at a consensus in case of the absence of a clear-cut picture. Serum samples were transferred into plain vials and stored at -20 to measure serum ferritin concentration using chemiluminescence assay using DXI 600 (Beckman Coulter, Inc., 250 S. Kraemer Blvd. Brea, CA 92821, USA) and soluble transferrin receptor (sTfR) assay using enzyme-linked immunosorbent assay (ELISA). Data were sourced daily from blood bank records and periodically from the departments of biochemistry and pathology investigation reports using a data collection pro forma.

Data collection and statistical analysis

All the data were collected using a pre-designed pro forma and entered into Microsoft Excel. The distribution of categorical variables such as gender and frequency of donations (<10 donations, 11–20 donations, and more than 20 donations), were expressed as frequency and percentages. The continuous variables such as age, complete blood count (CBC), serum ferritin, and sTfR assay were expressed as mean with standard deviation or median with range. The comparison of CBC, serum ferritin, and sTfR assay with donation frequency and time since the last donation was carried out using an independent student's *t*-test for two groups, otherwise one-way ANOVA. The Linear relationship of CBC, serum ferritin, and sTfR assay with age was carried out using correlation coefficient. All the statistical analyses were carried out at a 5% level of significance, and $P < 0.05$ was considered statistically significant. The statistical analysis was performed using SPSS for Windows version 20 (SPSS IBM Corp. Ltd. Armonk, NY, USA).

Statement of ethics

The Institutional Human Ethics Committee approved the study vide infra letter no JIP/IEC/2018/445 dated 16.11.2018. Informed consent for the donor's willingness to participate in the study was taken.

Results

A total of 323 nonremunerated regular blood donors were included in the study. The demographic and donation details of the donor population are summarized in Table 1. The mean age of the donor population was 30.8 years (standard deviation [SD] 8.1). The age range of the donors was between 18 and 60 years. There were only six female (2%) donors in the study. The mean height of the donors was 171.2 cm (SD 6.2) and weight was 76.5 kg (SD 12.3). The mean number of donations for the study population was 11.2 overall. For females, the median value was 6 (4–6). The maximum number of donations by a donor in the study group was 50.

For the purpose of the study, the participants were categorized into 3, donors with less than or equal to 10 donations, those who had donated between 11 and 20 times and those who had donated more than 20 times.^[7] All the hematological parameters with Serum Ferritin and sTfR assays were compared among the groups as summarized in Table 2. The CBC parameters were different among groups but were not statistically significant. The Ferritin and sTfR/Ferritin ratio expressed as sTfR/Log Ferritin and Log (sTfR/Ferritin) values were statistically significant between the groups.

The WHO hemoglobin cut-off criterion for indicating anemia is 12 g/dL for females and 13 g/dL for males.^[8] However, for the purpose of the study, we used a uniform Hb level of less than 12.5 g/dL as an anemic donor complying with the national cut off. A total of 15.2% of the study population had a ferritin level of <15 ng/ml. The relative percentage increased with donors who had donated more than 10 times with a factor of 2–3 times compared to those who had donated <10 times. The relative percentages are summarized in Figure 1.

The ferritin levels showed a negative correlation with the number of donations, as shown in Figure 2. The correlation coefficient was -0.27 , which was statistically significant, with a P value being <0.001 . There was also a statistically significant correlation between the sTfR/ferritin ratio and the number of donations with a correlation coefficient of 0.156 ($P < 0.01$).

Table 1: Demographic and donation factors of the study population

Factors	<i>n</i> =323, <i>n</i> (%)
Age (years)	
18-30	195 (60.4)
31-40	85 (26.3)
>40	43 (13.3)
Gender	
Males	317 (98)
Females	6 (2)
BMI	
<18.5	5 (1.5)
18.6-22.9	51 (15.8)
23-24.9	73 (22.6)
≥25	194 (60.1)
Number of donations (times)	
1-10	210 (65.2)
11-20	84 (26.1)
>20	28 (8.7)
Time since last donation (months)	
≤4	63 (19.5)
5-8	200 (61.9)
9-12	60 (18.6)

BMI: Body mass index

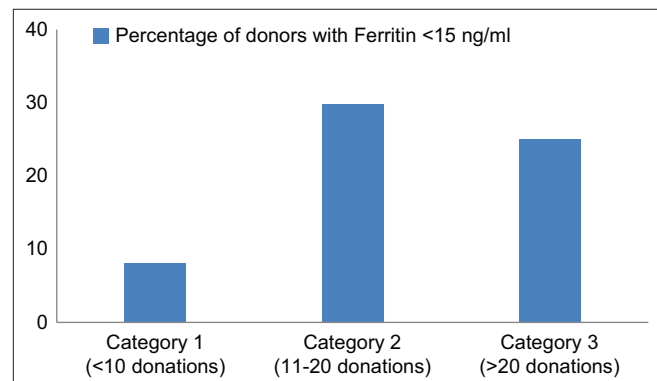


Figure 1: Percentage of donors with ferritin levels <15 ng/ml

The morphological picture seen on the peripheral smear classified based on size and color of the erythrocytes are summarized in Table 3. The microcytic hypochromic picture was seen in a higher number of individuals in Category II than Category I. However, it was not statistically significant ($\chi^2 = 1.7, P = 0.2$).

The prevalence of donors with indices less than the clinical cut-off defined by the Croatian Chamber of Medical statistics (HKMB) is summarized in Table 4.^[6] A total of 12.7% of the participants had an MCV of less than 83 fL. The proportion of such donors was significantly higher (21.4%) in donors who had donated more than 10 times with a $P = 0.02$.

No statistically significant correlation was noted between iron parameters and time since the last donation, as

shown in Figure 3. However, Hb levels showed a negligible positive correlation with ferritin levels with a correlation coefficient of 0.283 and were statistically significant [Figure 4].

Discussion

The screening test currently used to accept a blood donor for donation is the capillary (fingerstick) Hb estimation with $Hb \geq 12.5$ g/dL in both women and men in our country. Some countries have adopted a higher standard in men (e.g., 13.0 or 13.5 g/dl) that reflects the higher normal range for Hb in their population.^[9] The minimum Hb threshold is intended to prevent blood collection from donors with anemia, but it does not prevent blood collection from iron-deficient donors. Various laboratory tests have been used to assess the

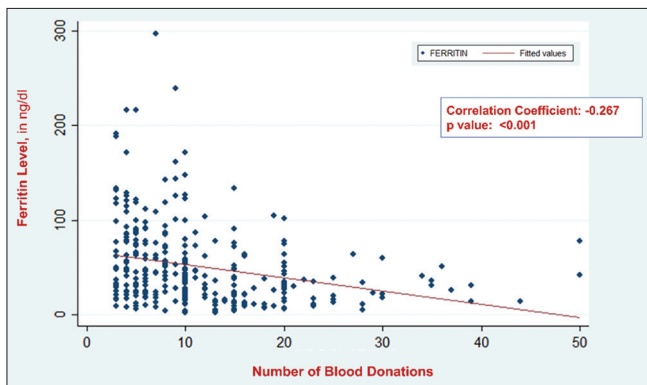


Figure 2: Scatter plot showing correlation between the serum ferritin level and the number of donations

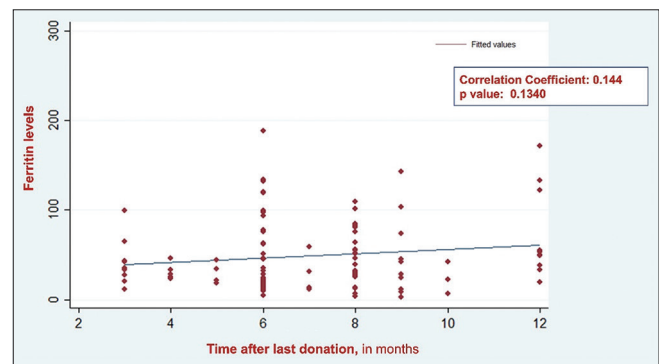


Figure 3: Scatter plot showing the correlation between Ferritin levels and time since the last donation

Table 2: Hematological and Iron parameters of the study population

	Mean (SD) or median (IQR)			P
	Category I (1-10 donations) (n=211)	Category II (11-20 donations) (n=84)	Category III (≥ 21 donations) (n=28)	
Hb (g %)	13.6 (1.4)	13.2 (1.4)	13.4 (1.8)	0.12
Hct (%)	40.8 (4.1)	39.2 (4.2)	40.2 (5.5)	0.12
MCV (fL)	91.2 (7.8)	89.4 (8.4)	91.7 (8.2)	0.19
MCH (pg)	29.1 (27.8-30.6)	28.9 (27-29.8)	28.7 (27.4-30.9)	0.57
MCHC (g/L)	31.66 (2.1)	31.65 (2.2)	31.65 (1.6)	0.99
RDW (%)	14.3 (1.5)	14.4 (1.6)	14.1 (1.7)	0.62
CCI	49.64 (23.1)	54.27 (23.97)	52.31 (16.4)	0.28
Retic count (%)	0.9 (0.6)	1 (0.6)	0.8 (0.5)	0.13
Ferritin (ng/ml)	60.8 (46.1)	36.2 (28.2)	29.8 (17.8)	<0.001*
sTfR/Log ferritin ratio	0.12 (0.27)	0.32 (0.74)	0.22 (0.49)	0.002*
sTfR/ferritin ratio (log sTfR/ferritin)	-2.8 (0.6)	-2.6 (0.9)	-2.5 (0.7)	0.003*

RDW: Red cell distribution width, CCI: Combined cell index, SD: Standard deviation, IQR: Interquartile range, Hb: Hemoglobin, Hct: Hematocrit, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: MCH concentration, sTfR: Soluble transferrin receptor

Table 3: Peripheral smear characters of the participants

Number of donations	Normocytic normochromic (n=311), n (%)	Normocytic hypochromic (n=1), n (%)	Microcytic hypochromic (n=11), n (%)
Category I (<10) (n=211)	204 (96.7)	1 (0.5)	6 (2.8)
Category II (11-20) (n=84)	79 (94)	0	5 (6)
Category III (>20) (n=28)	28 (100)	0	0

Table 4: Category wise comparison of prevalence with Croatian chamber of medical statistics^[6] clinical cut-offs for various red blood cell indices

	Category I (1-10 donations) (n=211), n (%)	Category II (11-20 donations) (n=84), n (%)	Category III (≥ 21 donations) (n=28), n (%)	Total (n=323), n (%)	χ^2 , P
MCV <83 (fL)	20 (9.5)	18 (21.4)	3 (10.7)	41 (12.7)	7.6, 0.02*
MCH <27.4 (pg)	44 (20.9)	24 (28.6)	7 (25)	75 (23.2)	2.1, 0.36
MCHC <32.6 (g/L)	145 (68.7)	55 (65.5)	24 (85.7)	224 (69.3)	4.2, 0.13
RDW >15%	149 (78.8)	60 (76.9)	22 (81.5)	231 (78.6)	0.3, 0.8
CCI >63.1	180 (85.3)	63 (75)	24 (85.7)	267 (82.7)	4.7, 0.1

RDW: Red cell distribution width, CCI: Combined cell index, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: MCH concentration

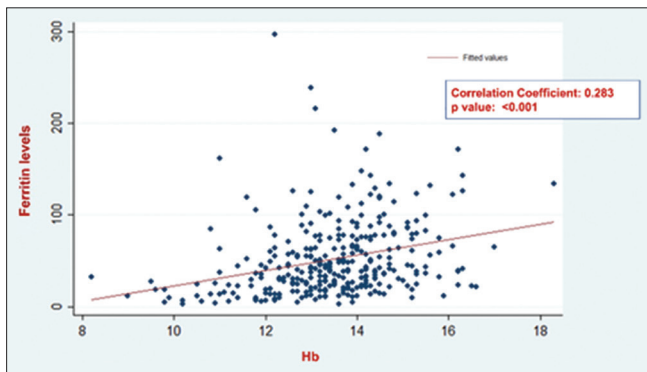


Figure 4: Scatterplot showing correlation between hemoglobin and ferritin levels

iron, namely ferritin, sTfR, sTfR/ferritin ratio, zinc (free erythrocyte), protoporphyrin (ZPP), red blood cell parameters, and more.^[6]

The pre-donation Hb cut off ≥ 12.5 g/dl, being similar for both men and women, which forms the basis of the current guidelines, is also debatable. A Hb level of 12.5 g/dL is higher than the lower limits of normal Hb levels for women and lower than the lower limits of normal Hb levels for men. Previous reports suggest that 10% of attempted whole blood donations had deferrals because of low Hb.^[10] Screening for Hb and iron indices helps predict donors at risk for subsequent anemia and helps in prevention strategies. Studies have shown that subclinical iron deficiency is prevalent among blood donors that meet Hb criteria for blood donation, which was observed in our study also.^[11]

At the stage of negative iron balance wherein the demand due to loss of blood exceeds the body's ability to absorb iron, the red cell morphology and indices remain normal, whereas the serum ferritin level is decreased. Hb synthesis remains unaffected as long as serum iron is within normal range. Marrow iron stores are said to be depleted totally when the serum ferritin is less than 15 $\mu\text{g/L}$. This stage is often called the latent or IDE stage. Once the levels fall below 15 $\mu\text{g/L}$, iron deficiency anemia sets in, and Hb and hematocrit begin to fall.^[7]

Transferrin receptors are present on the surface of RBC's and their synthesis increases when there is a reduction

of iron availability. These are shed into the blood and hence the name sTfR. When their levels are greater than the normal reference range, it suggests tissue iron deficiency in the absence of other conditions which increase sTfR levels, namely inflammatory conditions of the liver, thalassemia, hemolytic anemia, and higher altitude.^[12] However, this is unlikely to be relevant here as the donors are usually screened for these apparent conditions. Because of the lack of reference standard and assay variability, sTfR values are combined with ferritin measurements into a ratio of "log sTfR/ferritin. The log conversion makes the data more linear as the values had too much variability.^[2]

Both sTfR/Log Ferritin ratio and Log (sTfR/Ferritin) ratio have been used to distinguish IDE from storage iron depletion. Currently, not enough literature is available to compare/choose between them both. Castel *et al.* found that the transferrin (g/L)/log [ferritin ($\mu\text{g/L}$)] ratio is able to separate patients with ferritin <20 $\mu\text{g/L}$ (iron-deficient) from patients with ferritin >100 $\mu\text{g/L}$ (non-iron-deficient). The median Tf/log (ferr) ratio among the non-iron-deficient group was 0.84 in their study.^[13] In our study, the values were much lower, with a median of 0.22 range (0.12–0.32), because sTfR values obtained in our study were generally low and Ferritin was high with hardly values less than 20 in comparison to their study.

The estimation of log sTfR/ferritin has several advantages. Only two parameters are required, which reflect the functional iron compartment and correlate with depleted iron stores. An sTfR/log ferritin "index" has been utilized to distinguish storage iron depletion from IDE.^[2] Skikne and Cook demonstrated the advantage of using the log sTfR/ferritin construct and assessed iron stores quantitatively by carefully measuring iron loss in serial phlebotomy subjects bled until they became iron depleted.^[14,15] This method also allows estimation of tissue iron stores, which can be expressed as either iron surplus in stores or the iron deficit in tissues based on positive and negative values, respectively. It also permits an estimation of iron absorption in blood donors in longitudinal studies. In the REDS-II Donor Iron Status Evaluation (RISE) study, sTfR did not correlate with

IDE or plasma ferritin, R^2 0.54 versus R^2 -0.96. A log sTfR/ferritin value of 2.07 equated to a ferritin level of 26.7 $\mu\text{g/L}$ by multivariate regression, suggesting this ferritin level reflected IDE in healthy blood donors. At this threshold, ferritin had 95.1% sensitivity and 89.6% specificity in identifying IDE, and sTfR added little additional diagnostic information.^[16]

Radtke *et al.* and Nadarajan and colleagues used the 95th percentile log (sTfR/ferritin) in both males and females, resulting in a ratio of 2.5–2.6 to define IDE. This definition is less sensitive than the criterion used in RISE. This could have led to underestimating IDE's prevalence (8.2% prevalence reported in Radtke *et al.* and 10% in Nadarajan compared to 42% overall in RISE at the time of enrolment).^[17,18] However, the greater donation frequency in recruited RISE donors is also a factor contributing to the disparate prevalence.

Body iron can be determined from a small capillary blood specimen. The expression of body iron is based on body weight iron rather than absolute values. sTfR assay costs considerably more than other parameters.^[19]

Changes in conventional red blood cell morphologic parameters, including MCV, MCH, and MCHC, occur late in the development of iron depletion and are insensitive, resulting in low correlation with reduced iron levels and inferior to Hb in predicting subsequent Hb deferral.^[20,21] Serum ferritin levels are considered the gold standard for diagnosing iron deficiency in blood donors.^[22] Several studies report serum ferritin concentration as an indicator of iron stores.^[23,24] Previous reports state that serum ferritin <15 ng/ml is consistent with iron deficiency anemia, with 59% and 99% sensitivity and specificity, respectively.^[25]

Vuk *et al.* noted that CCI showed the highest degree of correlation with ferritin and a satisfactory diagnostic value of CCI in detecting depleted iron stores in blood donors.^[6] The mean CCI value in the normal population is noted to be 45–50 in the Croatian population, and our study showed similar values. The value is a reflection of the development of iron deficiency associated with MCH decline (hypochromia), MCV decline (microcytosis) and RDW increase (anisocytosis), and since in our study there was no significant difference was noted in those parameters same has been reflected in the CCI values with no difference among the groups.

RDW has been suggested as a marker for early diagnosis of iron deficiency compared to MCV or MCH.^[26] However, in our study, we did not find significant differences in RDW among the various donor groups as shown in the study by Ashish *et al.* a possible reason is that RDW is

increased in a more rapidly progressive iron deficiency and not in a chronic subclinical deficiency state.

In the present study, the prevalence of absent iron stores (serum ferritin <15 ng/ml) was 15.2%. Our results were similar to a study conducted in Pakistan where iron stores were estimated among 333 professional blood donors by serum ferritin were found to be 15%.^[23] A previous study conducted in Malaysia with 92 regular blood donors and 95 1st time blood donors found the prevalence of iron deficiency in 7.4% of all 1st time donors compared to 17.4% in regular donors.^[27] Mittal *et al.* also showed similar results by serum ferritin level less than 15 $\mu\text{g/L}$ in 21–29% of the donor population.^[28] Jeremiah and Koate got isolated iron deficiency 20.16% in 346 voluntary regular blood donors in Port Harcourt, Nigeria.^[29] The study conducted among 500 Spanish blood donors chosen at random and in 200 suitors for blood donation as a control group found blood donors showed increased iron deficiency (serum ferritin <15 ng/ml), 7.4% for men and 11.8% for women.^[30] Datta *et al.* state that donors potentially at risk for developing iron-deficiency anemia can be detected only by estimating ferritin.^[1]

The mean ferritin level in our study population was 40.4 ng/ml overall, with 41.4 ng/ml in men and 24.4 ng/ml in women, respectively. These observations reflect that the higher iron intake in male subjects and iron stores in women in the reproductive age group is always borderline and approximately 30% lower than men even with donating one unit of blood yearly.^[28] In men, there was an increase in serum ferritin levels with age. In previous reports, this increase was particularly marked between the ages of 18 and 30, when it may be assumed that the larger iron stores in men have been established.^[31] Iron stores in non-menstruating women compared with menstruating women improve despite older age, the greater number of lifetime donations, more pregnancies, and a greater frequency of donation. This emphasizes the significant contribution of menstrual blood loss to decreased iron stores. In a study conducted in Sokoto, Nigeria, iron stores assessed by serum ferritin found donors had lower serum ferritin, median 95 $\mu\text{g/l}$ than non-donors, median 136 $\mu\text{g/l}$.^[32]

Men have the most dramatic drop in ferritin level because of high iron stores before donation; women have a higher incidence of depleted iron stores, requiring re-evaluation of donation standards. This can be attributed to the fact that there is a high donation frequency of the male donors, and only male donors with low Hb values were treated, which explains the insufficient supplementation strategy. Re-evaluation of iron prophylaxis for men is required in addition to females. To assure compliance by the donor, iron supplementation therapy needs

individual information. Those donors with absent iron stores (<15 ng/ml) should be considered for iron supplementation of 100 mg daily during the 20 days after donation. Rapid improvement in Hb has been found in donors with ferritin <15 ng/ml who received iron treatment.^[33]

In our study, serum ferritin levels gradually decreased according to the number of donations which was statistically significant. The difference was evident comparing regular donations with less than ten donations and those with more than 10. After that, ferritin levels remain constant with 20 and above donations. This observation was similar to a study conducted among blood donors in Sokoto, Nigeria.^[32] However, this finding was variance with observations by Vilzu and co-workers who found no significant difference between ferritin levels in controls and donors donating less than 20 units.^[34] A 14-fold increase in the risk of iron deficiency in donors donating 3–4 units in the preceding 2 years and 50-fold increase in those who donated ten or more units compared to 1st time donors found in the RISE study.^[35] The study conducted in Malaysia with 95 regular blood donors and 95 1st time donors found a prevalence of iron deficiency in 7.4% of all 1st time donors compared to 17.4% in regular donors.^[36] To determine iron stores before depletion, Ferritin measurement should be taken at an even earlier stage. For donors who are on regular iron supplementation, serum ferritin measurement after every 10th donation is sufficient to trace the efficacy of therapy. In our study, 39.1% of anemic donors had ferritin 15–50 ng/ml, whereas 48.3% non-anemic donors had ferritin 15–50 ng/ml. 86.7% of the individuals with Hb more than 12.5 have ferritin levels <100 ng/dl, which was statistically significant. This provides evidence for regular ferritin screening and regular iron supplementation strategy. Reduction in allowed frequency of donation to a lower number, such as 4 times a year, as found in the current recommended federal regulations, appears to be an appropriate strategy. Nevertheless, this approach does not provide adequate protection to menstruating women and restricts men and non-menstruating women unduly. Previous reports suggest that donating 2–3 units per year provided pre-donation Hb and ferritin value ≥ 14.7 g/dL and 58.9 $\mu\text{g/L}$.^[37]

Iron depletion begins with the gradual loss (a relatively small compartment) of storage iron. With the poor correlation of hemoglobin with the iron status being established, several markers are being investigated to be adopted for the same. Few of the risk factors identified include female donors of reproductive age group, or plasma ferritin <12 ng/ml, 1st time and reactivated female donors, those who have donated ten or more

times are associated with more risk of developing iron deficiency.^[16] Currently, Hb and in some institutes, CBC are the only parameters relied on for donor acceptance. With no indication whatsoever with these tests regarding iron status, this study shows that iron stores can be becoming diminished unnoticeably. Ferritin and sTfR/Ferritin ratio would help assess at least the above-mentioned high-risk category for iron deficiency. However, the logistics for adopting the same in a blood center needs to be rigorously thought and sorted.

The strength of our study is that we got an adequate sample size of 323 regular blood donors with complete CBC, serum Ferritin levels, and peripheral smear findings. sTfR and the ratio were attempted in the study, which not many studies from India have reported. Comparing the number of donations with donor iron status helped identify the population at risk and consider further implementing potential predictive methods to estimate the risk of iron deficiency in individual donors.

sTfR assay determination was performed using ELISA. Because of the lack of reference standard could not be compared with other studies as the results vary according to the assay used.

Our study found that regular blood donors had low iron stores (decreased serum ferritin is seen in 15.2% of donors). Using the current guidelines (Hb >12.5g/dL) for donation, the donor's actual iron status is not reflected. This envisages a need to review screening tests for the selection of blood donors.

Conclusion

Ferritin determination helps find those individuals who may not be iron deficient by laboratory criteria but have decreased iron stores and are likely to develop an iron deficiency if bled. Therefore, measuring serum ferritin in addition to Hb will protect the donors and ensure safer blood donation. It seems clear that some individuals who are bled are already iron deficient, although not overtly anemic.

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Conflicts of interest

There are no conflicts of interest.

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