Estimation of Serum Ferritin Levels in Blood Donors and its Association with Frequency of Blood Donation and Blood Indices

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ABSTRACT

Introduction: Blood donation is one of the most significant contributions that a person can make towards the society. A donor generally donates maximum 450 mL of blood at the time of donation. If 450 mL of blood is taken in a donation, men lose 242±17 mg and women lose 217±11 mg of iron. Hence, adequate iron stores are very important in maintenance of the donor's health.

Aim: To assess the influence of frequency of blood donation on iron levels of blood donors by estimating Haemoglobin (Hb) and other blood indices which reflect iron status of blood and serum ferritin which reflects body iron stores.

Materials and Methods: The present study was a cross-sectional analytical study, conducted on 150 blood donors, 18-40 years of age presenting to the Blood Bank in Government Medical College, Kottayam, Kerala, India, between December 2016 to December 2017. Total of 150 donors were divided into four groups according to the number of donations per year. Group I were the first time donors with no previous history of blood donation, Group II- included those with history of donation once in the previous year, Group III- those donors with history of donation twice in the previous year and Group IV- those having history of donation thrice in the previous year. Six mL of whole blood collected from each donor, two mL was used for estimating Haemoglobin (Hb), Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Hb (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC) in haematology analyser. Serum separated from remaining four mL of blood underwent ferritin analysis by Chemiluminescence Immunoassay (CLIA) method. Iron stores were considered normal at serum ferritin value from 23.9-336 ng/mL in males and 11-307 ng/mL in females. Statistical analysis was performed in Statistical Package for the Social Sciences (SPSS) version 16.0. Analysis of Variance (ANOVA) test and Pearson's correlation test were used to find association between various parameters and collected data. The p-value <0.05 was considered as statistically significant.

Results: There was no significant correlation between serum ferritin level and frequency of blood donation. MCH, MCHC showed significant association (p-value=0.039 and 0.007, respectively) with frequency of blood donation. Low positive correlation was seen between Hb and PCV with serum ferritin levels (r=0.381, p-value <0.001 and r=0.354, p-value <0.001, respectively).

Conclusion: There is no significant association between frequency of blood donation and serum ferritin levels.

INTRODUCTION

Blood donation is one of the most significant contributions that a person can make towards the society. Human beings inspite of all the scientific and technological progresses have so far failed to find a proper alternative for blood. Therefore, there is no other option for patients to receive blood from anywhere else than from other people, thus blood donation is called "life donation". On 14th June, every year, the world blood donor day is declared. A donor generally donates maximum 450 mL of blood at the time of donation. If 450 mL of blood is taken in a donation, men lose 242±17 mg and women lose 217±11 mg of iron [1,2]. Adequate iron stores are very important in maintenance of the donor's health [3]. It is not only important to give a safe blood donation to the recipient but it is also important that the donor is safe after donation [4]. 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 at a time when the demand of blood is soaring all over the world and the donors are becoming scarce [5,6].

Chronic iron deficiency is a well recognised complication of regular blood donation [7]. After a single donation, a person needs approximately three months to replenish iron stores [8]. In the majority of blood banks, Haemoglobin (Hb) measurement is used as a screening test for the ability to donate blood. Since, Hb levels may be normal in the presence of reduced iron stores, the use of this parameter has been reported to have poor sensitivity in the detection of early stages of iron deficiency [3]. Indeed, an accurate diagnosis of a state of iron deficiency requires several laboratory tests. Measurements of serum ferritin concentrations and red cell indices such as MCV and MCH can be used with a high degree of accuracy and precision [9]. Previous studies have shown that the serum ferritin levels were reduced markedly in regular donors corresponding to annual donation frequency [10]. In order to fulfill the global and national drive to recruit and retain regular repeat voluntary blood donors, the iron status of the donors needs to be identified and necessary steps for iron supplementation need to be taken [11]. Iron is an important dietary mineral and is essential for our body to function normally. When whole blood is donated about 200-250 mg of iron is lost with each donation [12,13]. Generally, the body will replace any iron loss through diet and it is recommended to have a healthy dietary intake of iron-rich foods. However, replacing this amount of iron through diet alone can take some time. This means the iron stores may decrease over time and may lead to iron deficiency [14].

Regular blood donors undergo a progressive decline in iron reserves, while some develop frank iron deficient erythropoiesis. The prevalence of iron depletion is significantly higher in menstruating women and increases progressively as the rate of donation increases [15]. Minimal daily iron supplementation was beneficial in maintaining body iron stores and Hb levels in menstruating females [16]. Iron deficiency is a significant cause of deferral in people wishing to donate blood. If iron removed from the body is not replaced, then donors may become iron

deficient [17]. Serum ferritin would need to be included in assessment of blood donors, particularly among regular male donors and all female donors so that a better picture of iron status may be obtained and donors managed and counselled appropriately. The reduction of iron in donors is not sufficiently compensated by a normal diet and it is to be discussed whether iron supplementation after donations will restore the iron status more quickly [18-20].

A safe blood donation contains approximately 500 mL of whole blood and can help more than three patients. One of the general and common conditions of a safe donation in the world is that donors should have sufficient amount of iron in their bodies. So, the present study was aimed to assess the influence of frequency of blood donation on iron levels of blood donors by estimating Hb and other blood indices which reflect iron status of blood and serum ferritin which reflects body iron stores.

MATERIALS AND METHODS

The present study was a cross-sectional analytical study conducted on blood donors presenting to the Blood bank in the Department of Transfusion Medicine, Government Medical College Kottayam, Kerala, India, between 31st December 2016 to 31st December 2017, after obtaining the approval from Institutional Review Board (IRB No.126/2016).

Sample size calculation: The sample size was calculated, as was suggested by Mittal R et al., [1].

$$N=\frac{Z^2 \times SD^2}{d^2}$$

The calculation came out to be 132, where N=sample size, Z=1.96 at 5% level of significance, SD=standard deviation: 25.85, d=absolute precision; 17% of SD. Considering 10% non-response, sample size was taken as 150.

Inclusion criteria: All donors between the age group of 18-40 years during one year period and weighing more than 45 kg were included in the study after obtaining the informed consent.

Exclusion criteria: Donors with Hb <12.5 g%, or those with history of smoking, alcoholism, systemic illness like diabetes and hypertension or with history of blood loss in the past one year by surgery/accident or medical conditions like menorrhagia, donors with liver disease, malignancy, inflammatory conditions, thyroid disorders and the ones unwilling to participate in this study were excluded from the study.

The total sample of 150 selected donors, and all relevant details-demographic details, history of smoking and alcohol intake were noted. In the present study, the total sample was divided into four groups according to the number of donations per year-group I were the first time donors with no previous history of blood donation, Group II were the donors who had previously donated once in the previous year, group III were the donors who had donated twice in the previous year, group IV were the donors who had donated thrice in the previous year [Table/Fig-1].

No. of blood donations in past one year	Frequency	Percent
Group: 1-1st time donor	37	24.7
Group: 2-2nd time donor	37	24.7
Group: 3-3rd time donor	38	25.3
Group: 4-4th time donor	38	25.3
Total	150	100.0

[Table/Fig-1]: Distribution of study population based on number of blood donations in the past one year in groups.

A six mL of whole blood was collected from each donor during process of blood donation. Out of six mL of the blood, two mL was collected in Ethylene Diamine Tetra Acetic Acid (EDTA) tubes which were used for estimating Hb, PCV, MCV, MCH, MCHC while four mL collected in plain tubes for serum ferritin analysis.

Estimation of Blood Parametres

These test were performed in Sysmex XS-1000i haematology analyser on whole blood anticoagulated with potassium EDTA. Haematocrit (HCT) is measured as the ratio of the total Red Blood Cell (RBC) volume to whole blood using cumulative pulse height detection. Hb is converted to Sodium Lauryl Sulfate (SLS)- Hb and read photometrically.

SLS is the RBC lysing reagent that releases the Hb to be measured by SLS Hb method. Its active ingredient was Sodium Lauryl Sulfate 1.7 g/L and is stored at controlled temperature of 2-30°C, with stability for 60 days.

Reagents used: Sysmex Cellpack Ethanol Extract of Pinus Koraiensis Leaves (EPK) with ingredients Sodium Chloride 6.38 g/L, Boric Acid 1.00 g/L, Sodium Tetraborate 0.20 g/L and EDTA-2K 0.20 g/L, Stored at a controlled temperature of 5-30°C, was used as whole blood diluent for use in the determination of Hb and impedance counting and sizing of blood cells. It also forms a laminar sheath flow around the diluted sample for hydrodynamic focusing of the RBCs and platelets.

Sysmex stromatolyser- Differential Lysing Reagent 4DL Product Code by Manufacturer FFD-200A (FFD) is the lysing reagent and diluent for the enumeration of neutrophils, lymphocytes, monocytes, eosinophils, and basophils after eliminating RBC stroma. Its active ingredients include non-ionic surfactant 0.18% and organic quaternary ammonium salts 0.08% and is stored at a controlled temperature of 2-35°C, with a stability for 60 days.

Dying Solution 4DS Product Code by Manufacturer FFS-800A (FFS) is used to stain leukocytes in diluted, lysed blood samples for the determination of the 5-part differential including neutrophils, lymphocytes, monocytes, eosinophils and basophils. Its active ingredients were ethylene glycol 96.9%, methanol 3.0% and polymethine dye 0.002% and were stored at controlled temperature of 3-35°C with stability for 90 days.

Calibration: Calibration is done using Sysmex Sysmex Corporation System (SCS)-1000 Calibrator. The ranges considered as reference for this study were: Hb-13.6-16.7 g/dL in males and 11.8-15.1 g/dL in females, PCV-41-50% in males and 36-46% in females, MCV- 82-99 fL, MCH-27-32 pg and MCHC-32-36 g/dL.

Estimation of Serum Ferritin

Four mL of blood of each donor was collected in plain tubes and serum was separated by centrifugation at 3000 rpm for 10 minutes and was kept at -20°c. The sample underwent CLIA by Beckman Coulter Access 2 Immunoassay system. Chemiluminiscence is the emission of light when an electron returns from an excited or higher energy level to a lower energy level. The excitation event is caused by a chemical reaction; caused by oxidation of an organic compound.

Reagent used: Access ferritin reagent containing paramagnetic particles coated with goat antimouse IgG: mouse antiferritin complexes, Bovine Serum Albumin (BSA) in Reagent 1a (R1a) and Goat antiferritin alkaline phosphatase (bovine) conjugate, BSA, protein (goat, murine) in R1b.

Calibration: Calibration is done using access ferritin calibrator with following references:

S0: buffered BSA matrix

S1-S5: human liver ferritin, buffered BSA matrix

S0-0 ng/mL, S1-10 ng/mL, S2-50 ng/mL, S3-200 ng/mL, S4-500 ng/mL, S5-1500 ng/mL.

Iron stores were considered normal at serum ferritin values from 23.9-336 ng/mL in males and from 11-307 ng/mL in females, hence these were considered as reference ranges for the present study.

STATISTICAL ANALYSIS

Statistical data was entered in Microsoft Excel and statistical analysis was performed in SPSS version 16.0. Microsoft Word and Excel

were used to generate tables. All the variables were analysed and compared between the four groups of study sample. Continuous data are reported as mean±Standard Deviation (SD). ANOVA test was performed to find association between frequency of blood donation and blood parameters. Pearson's correlation coefficient was used to find association between serum ferritin levels and other blood parameters. Significance is assessed at 5% level and a p-value <0.05 was considered as statistically significant.

RESULTS

Mean age of the study population was 27.53±6.873 years. (Range: 18-40 years, with majority (95.3%) being males [Table/Fig-2]. Males showed a mean ferritin level of 88.351 mg/mL and females showed a mean ferritin level of 26.686 ng/mL. All the study participants were taking a non-vegetarian diet and none gave the history of major surgery/blood loss in the past one year. None among the females had child birth in the past two years and none reported menorrhagia. Their past histories regarding smoking and alcoholic habits is shown in [Table/Fig-2].

Parameters	Frequency	Percent
Age group (years)		
<20	27	18.0
21-30	70	46.7
31-40	53	35.3
Gender		
Male	143	95.3
Female	7	4.7
Total	150	100.0
History of smoking		
Present	13	8.7
Absent	137	91.3
History of alcohol intake		
Present	42	28.0
Absent	108	72.0
Total	150	100.0

[Table/Fig-2]: The demographic details and clinical past history of all subjects.

Among those who donated blood more than once in the past one year (n=113), mean interval between the donations was 5.22±1.450 months. The mean values of laboratory investigations and blood parameters of donors is shown in [Table/Fig-3].

Parameter	Minimum	Maximum	Mean	SD
Hb (g/dL)	7.2	17.9	14.187	1.4412
PCV (%)	28.1	53.8	41.622	3.8324
MCV (fl)	54.4	94.9	83.917	5.3350
MCH (pg)	13.8	35.1	28.620	2.4034
MCHC (g/dL)	25	40	34.12	1.717
S. Ferritin (ng/mL)	2.9	935.6	85.473	103.3824

[Table/Fig-3]: Blood Parameters of the study population.
Hb: Haemoglobin; PCV: Packed cell volume; MCV: Mean corpuscular volume; MCH: Mean corpuscular haemoglobin; MCHC: Mean corpuscular haemoglobin concentration

Mean serum ferritin value for donors (first, second, third and fourth) is shown in [Table/Fig-4]. There was no significant correlation between serum ferritin level and frequency of blood donation in this study.

The p-value of MCH, MCHC is less than 0.05 and showed significant association with frequency of blood donation. Hb, PCV, MCV did not show any significant association with frequency of blood donation [Table/Fig-5].

Pearson correlation coefficient was used to compare serum ferritin levels with other blood parameters. Low positive correlation was seen between Hb, PCV with serum ferritin levels. This was found to

No. of blood donations in past one year	Mean	Std. Deviation
Group 1 donor	82.581	53.3841
Group 2 donor	84.535	87.5618
Group 3 donor	78.513	89.7140
Group 4 donor	96.163	156.7893
Total	85.473	103.3824

[Table/Fig-4]: Mean ferritin level (ng/mL) based on number of blood donations in the past one year.
According to ANOVA test, F value=0.200; p-value=0.896

Parameter	No. of donations	Mean	Std. Deviation	F-value	p-value
Hb g/dL (gram per decilitre)	First time	14.035	1.5406	0.378	0.769
	Donated once	14.108	1.2291		
	Donated twice	14.366	1.2622		
	Donated thrice	14.234	1.7111		
	Total	14.187	1.4412		
PCV % (percentage)	First time	41.546	4.4413	0.934	0.426
	Donated once	41.000	3.3267		
	Donated twice	41.476	3.1037		
	Donated thrice	42.447	4.2942		
	Total	41.622	3.8324		
MCV fL (femtolitre)	First time	83.768	3.8671	0.889	0.449
	Donated once	85.057	4.6932		
	Donated twice	83.787	5.1552		
	Donated thrice	83.082	7.0714		
	Total	83.917	5.3350		
MCH pg (picogram)	First time	28.273	1.1654	2.859	0.039
	Donated once	29.281	2.0411		
	Donated twice	29.045	2.6662		
	Donated thrice	27.889	3.0803		
	Total	28.620	2.4034		
MCHC g/dL (gram per decilitre)	First time	33.78	1.110	4.232	0.007
	Donated once	34.43	1.667		
	Donated twice	34.73	1.862		
	Donated thrice	33.53	1.886		
	Total	34.12	1.717		

[Table/Fig-5]: Table showing mean ferritin level based on number of blood donations in past one year.
F-values and p-values calculated according to the ANOVA test. The p-value <0.05 to be considered significant. Hb: Haemoglobin; PCV: test. The p-value <0.05 to be considered significant. Hb: Haemoglobin; PCV; Packed cell volume; MCV: Mean corpuscular volume; MCH: Mean corpuscular Hb; MCHC: Mean corpuscular haemoglobin concentration

be statistically significant with a p-value <0.001. Though negligible correlation was seen between serum ferritin and MCV, MCH, this was found to be statistically significant [Table/Fig-6].

Parameter	Pearson correlation coefficient®	p-value
Hb	0.381	<0.001
PCV	0.354	<0.001
MCV	0.209	0.010
MCH	0.228	0.005
MCHC	0.116	0.156

[Table/Fig-6]: Blood indices of the study subjects with their correlation with serum ferritin according to Pearson Correlation Coefficient.
p-value <0.05 to be considered significant. p-value <0.001 considered Low positive correlation (Significant); p-value 0.010 Negligible correlation (significant); Hb: Haemoglobin; PCV: Packed cell volume; MCV: Mean corpuscular volume; MCH: Mean corpuscular haemoglobin; MCHC: Mean corpuscular haemoglobin concentration

DISCUSSION

The present study compared serum ferritin levels with frequency of blood donation, Hb levels and other blood indices (MCV, MCH,

MCHC). They showed a mean serum ferritin level of 85.473 ng/mL. This study shows that there was no significant relation between frequency of blood donation and serum ferritin levels. This was in contrast to studies conducted by Mittal R et al., and Mahida VI et al., [1,21]. Considering distribution of study population based on number of blood donations during life time 37 donors who were first time donors, had a mean ferritin level of 82.581 ng/mL. The present study did not showed any significant relation with total number of blood donations during life time. This was in contrast to the study conducted by Simon TL et al., which showed that 8% of men and 38% of women have reduced iron stores, as assessed by serum ferritin status, after five donations [22]. In the study by Milman N and Kirchhoff M, 26% of regular blood donors had low ferritin levels and 12% were found to be anaemic [11]. In Nigeria, in 1990, Usanga EA observed that the mean ferritin concentration of 64.75±4.6 ng/mL in first time donors was significantly higher than the mean value of 49.19 ng/mL among frequent blood donors suggesting that some blood donors may have pre-latent or latent iron deficiency at the time of donation and become iron deficient after blood donation [23].

In Adediran A et al., study the mean serum ferritin level was significantly lower in the regular donors than in the first time donors [24]. Szymczyk-Nuzka M and Wołowiec D when determining the prevalence of iron deficiency in regular blood donors noted a lower serum ferritin in this group in contrast with first time donors who had normal serum ferritin levels [25]. Norashikin J et al., also reported significantly lower serum ferritin levels in regular blood donor [26]. Several investigators have noted a significant reduction in ferritin levels with increasing donations [27-29]. Contrary to these published studies, this study did not show any significant association between serum ferritin levels in first time donors and regular donors.

Though MCH, MCHC showed statistically significant relation with frequency of blood donation, they are not clinically relevant. Group 1 donors showed a mean MCH of 28.273 fL and Group 4 donors showed a mean MCH of 27.889 fL. Similarly, Group 1 donors showed a mean MCHC of 33.78 g/dL and group 4 donors showed a mean MCHC of 33.53 g/dL. These findings correlated with study conducted by Tailor HJ et al., [5]. There was a significant correlation between the frequency of donations, last donation interval and blood indices in the study conducted by Tailor HJ et al., [5]. A positive correlation was seen between Hb and ferritin levels and PCV and ferritin levels. This was in correlation with study conducted by Suominen P et al., [30]. Also, a significant correlation was seen between MCV and ferritin levels and MCH and ferritin levels. This similar finding is obtained in study conducted by Abdullah SM [6].

Considering distribution of study population based on gender 95.3% were males and 4.7% were females. Males showed a mean ferritin level of 88.351 mg/mL and females showed a mean ferritin level of 26.686 ng/mL. Here, there was no statistically significant relation between frequency of blood donation and serum ferritin levels. In the study conducted by Deepa DP et al., distribution on the basis of number of donations per year and serum ferritin <15 ng/mL in male donors were 6.9 % in first time, 19.4 % in once a year, 26.7% in twice a year and 50% in thrice year donation [31]. Among female donors 40.7% in first time, 50% in once a year, 50% in twice a year donation had serum ferritin levels <15 ng/mL and hence there was a definite correlation between dwindling of serum ferritin level and the frequency of donation [31].

Also, sample size was 150 as compared to 400 in the study conducted by Mittal R et al., [1]. Rosvik AS et al., recommended that donors with serum ferritin levels below 50 µg/L should be given iron while donors with levels above 80 µg/L needs no additional iron [32]. There are no formal recommendations in standards or regulations for the use of iron supplementation after blood donation, but dietary advice is a relatively widespread practice in donor centers. Even casual use of iron supplementation has shown to be advantageous to blood donors [33].

The blood donors at risk of developing iron deficiency can be detected only by the estimation of the levels of serum ferritin levels. The iron status of the donors should be identified and necessary steps for iron supplementation need to be taken to fulfill the national and global drive for the blood requirements.

Here, contrary to previous studies, a statistically significant correlation was not obtained between serum ferritin levels and frequency of blood donation. This may due to difference in socio-economic status, diet, sample size etc. All the donors belonged to Kottayam district of Kerala, middle class or higher middle class family consumed red meat most days of the week and made sample size to 150. Also, total number of life donations was less than 10 in majority of the donors. So, this study shows that depletion of iron stores is not a concern for low frequency donors and blood donation should be encouraged in our society.

In this study, there was no statistically significant relation between Hb levels and frequency of blood donation. This was similar to the study by Abdullah SM, Finch CA et al., and Yousefinejad V et al., [6,13,34]. In the study, by Milman N and Kirchhoff M, the study population was large and they have categorised the ferritin levels as decreased and depleted [11]. Study done by Norashikin J et al., took into consideration a period of two years for assessing the frequency of donation [26], whereas, in this study the groups were categorised using one year for the frequency of blood donation.

In the study, conducted by Mahida VI et al., there was no significant difference between ferritin levels in controls and donors donating less than 20 units [21]. Akpotuzor J et al., reported that there was no observable difference in biochemical iron parameters between regular donors and normal controls [35]. This was similar to the results obtained in this study. In contrast, several other studies have documented lower serum ferritin levels in regular blood donors compared with healthy controls [31,32].

Limitation(s)

In the present study, iron stores were estimated based on serum ferritin levels. Other parameters indicating iron status such as, serum iron, transferrin saturation, Total Iron Binding Capacity (TIBC) were not included. It was a small sample study with all subjects belonging to Kottayam Kerala, specifically, which might be a factor affecting measurement of blood indices.

CONCLUSION(S)

Blood donation is an act of benevolence rather than altruism. Both the donor and recipient gains. The study showed no significant relation between frequency of blood donation and serum ferritin levels and positive correlation between serum ferritin levels and Hb, MCH, MCHC showed significant relation with frequency of blood donation. The study data suggested that ferritin levels, Hb concentration, PCV are not significantly affected by regular blood donation. A larger study may be needed to confirm these preliminary findings. This study shows that a person can donate blood three times a year without being concerned about depletion of iron stores and blood donation should be encouraged in our society.

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