ORIGINAL RESEARCH

Utility Of Estimation Of Serum Ferritin In Blood Donors: A Cross – Sectional Study

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ABSTRACT

Introduction: Chronic iron deficiency is a well-recognized complication of regular blood donation [1]. 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 may be detected by serum ferritin estimation [1]

Materials and methods: The prospective, cross-sectional study was carried out on blood donors who had donated twice or thrice previously in a year and were donating voluntarily for the third or fourth time in blood bank of BLDEU Shri B.M. Patil Medical College, Hospital and Research Centre, Bijapur from October 2013 to March 2015.
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-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 which was 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 underscores the utility of Serum Ferritin level estimation as an adjuvant to hemoglobin assessment in multiple time donors. While there is clear cut consensus among blood banks to consider hemoglobin as a method for blood donor anemia screening, serum ferritin estimation can be planned before bleeding a prospective non anemic multiple time donor to correctly assess the iron status.

Keywords: Serum ferritin, voluntary donors, repeat blood donors, hemoglobin.

INTRODUCTION

With each donation men lose 242 ± 17 mg and women lose 217 ±11 mg of iron which implies that regular blood donation may lead to chronic iron deficiency. A minimum of three months is required as this is the period in which hemoglobinization of RBCs take place and required mean hemoglobin concentration is achieved after blood donation so a healthy individual can donate blood up to four times a year [1]. Cut-off value of hemoglobin of 12.5 g/dl is used as a criterion for blood donation [2]. Increasing donation frequency has been shown to be associated with a distinct fall in iron stores [3]. Majority of blood banks use hemoglobin and/or hematocrit measurements as routine screening tests for blood donation [2, 4]. However, such routine methods do not necessarily reflect the status of the total body iron content of an individual which falls with increasing number of blood donations as stated earlier [2]. To know the iron status and identify the donors potentially at risk for developing iron deficiency, serum ferritin (SF) estimation can be done. This becomes more important when hemoglobin levels are normal and not correlating with reduced iron stores[1,5].Ferritin is found mainly in the reticulo-endothelial cells of liver, spleen and bone marrow and is the main iron storage compound in the body. Ferritin (31-300µg/l) is present in the circulating plasma and reflects the iron stores. Therefore, ferritin can be used as a sensitive index to detect the earliest stage of iron deficiency [2]. According to World Health Organization (WHO) serum ferritin concentration <15 ng/ml indicates depleted iron stores in those >5 years of age [6].

AIMS AND OBJECTIVES

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, the present study aims to determine the utility of estimation of serum ferritin in blood donors to identify the donors in the stage of depleted iron stores before they develop iron deficiency anemia.

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

MATERIALS AND METHODS

Study design and donors (figure I)**:**

Type of Study: It's a prospective cross-sectional study.

Place of study and Study Subjects: The study was carried out on blood donors who were donating blood voluntarily in the blood bank of BLDEU Shri B.M. Patil Medical College, Hospital and Research Centre, Bijapur after taking Ethical clearance from the institute.

Study Duration: It was a time bound study with study period of one year from October 2013 to September 2014.

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

Exclusion criteria: Donors who did not fulfill standard blood donor selection criteria were excluded from the study.

Methodology:

1. Pre-donation hemoglobin estimation was done using point of care portable Hemo Cue Hb photometer based on spectrophotometry.
2. Donors were screened on the basis of routine blood donor selection criteria.
3. After blood donation of 350/450 ml of whole blood, additional samples were collected in plain and EDTA vacutainers.
4. The samples were processed within 4-6 hours of collection.
5. EDTA blood was tested using the 5-part automated Hematoanalyzer Sysmex XN series and routine haematological parameters like Hb, haematocrit, red cell count, MCV, MCH, MCHC, RDW of the sample were measured.
6. Serum from plain vacutainer without any anticoagulant was used for Serum ferritin estimation using ELISA technique.

Study Design: Donors were broadly divided into 2 groups, Group A (male and female) and Group B (male and female), based upon whether they were donating for the first time with no previous donation or had previously donated blood on one or more occasions (Figure: 1). Group A was control group as the donors had never donated before and were donating for the first time. Group B was further divided into 3 subgroups – group I, II, and III based upon the increasing frequency of donation. All the donors had hemoglobin value >12.5gm/ dl.

All the donors in different groups and subgroups were analyzed in terms of Hb, hematocrit, MCV, MCH, MCHC, RDW and serum ferritin. Comparison of mean of all parameters between group A and B was done among male and female donors, and Inter-Group comparison of mean values of Serum Ferritin (ng/ml) levels by number of donations was done.

Statistical analysis: All characteristics were summarized descriptively. For continuous variables, the summary statistics of N, arithmetic mean (referred to as mean), standard deviation about the arithmetic mean (SD) were used.

For continuous data, the differences of the analysis variables were tested with the t-test. If the p-value was > 0.05, then the results will be considered to be not significant and if p-value was < 0.05 then results would be considered to be statistically significant. Data was analysed using SPSS software version 16.

RESULT

Table 1: 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)	80.08	62.65	48.34	40.83	**0.0001***
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

Table 2: 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)	39.58	31.83	19.62	18.41	0.05
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

Table 3: Intergroup comparison of Serum Ferritin in male donors

	MEAN	**SD**		**MEAN**	**SD**	**P VALUE**
GROUP A	80.08	62.65	**GROUP B**	48.34	40.83	<0.05
GROUP A	80.08	62.65	**GROUP IB**	53.04	42.18	<0.05
GROUP A	80.08	62.65	**GROUP IIB**	44.99	41.09	<0.05
GROUP A	80.08	62.65	**GROUP IIIB**	42.74	38.98	<0.05
GROUP IB	53.04	42.18	**GROUP IIB**	44.99	41.09	>0.05
GROUP IB	53.04	42.18	**GROUP IIIB**	42.74	38.98	>0.05
GROUP IIB	44.99	41.09	**GROUP IIIB**	42.74	38.98	>0.05

Table 4: Comparison of serum ferritin levels based on the frequency of donation for male

Number Of Donations	**Serum Ferritin In Ng/Ml**	**Sd**	**Range**
	Mean		
0 (Group A)	80.08	62.65	12-262
1(Group IB)	53.04	42.18	6-172
2(Group IIB)	44.99	41.09	1.2-107
Multiple donations over lifetime (Group IIIB)	42.74	38.98	2.7-167

Table 5: Comparison of serum ferritin levels based on the frequency of donation for female

Number Of Donations	**Serum Ferritin In Ng/Ml**	**Sd**	**Range**
	Mean		
0 (Group A)	39.58	31.83	5.6-171
1(Group IB)	21.33	18.97	2.1-53.99
2(Group IIB)	5.2	NA	NA
Multiple donations over lifetime (Group IIIB)	20.85	25.67	2.7-39

Table 6: Distribution of donors on the basis of number of donations per year and serum ferritin < 15 ng/ml in total number of donors within a group, male and female donors

number of donations per year	**Donors with serum ferritin <15 ng/ml (total donors)**	**%**	**Male donors with serum ferritin <15 ng/ml (total donors)**	**%**	**Female donors with serum ferritin <15 ng/ml (total donors)**	**%**
0	6 (133)	4.51	3 (80)	3.75	3 (53)	5.66
1	10(53)	18.86	7(46)	15.22	3(7)	42.86
2	8 (17)	47.06	7 (16)	43.75	1 (1)	100
Multiple donations over lifetime	11(31)	35.48	10(29)	34.48	1(2)	50

Table 7: Distribution of donors based on gender and serum ferritin

Sex	donors with serum ferritin <15 ng/ml (Total Donors)	%
Male	27 (171)	15.79
Female	8 (63)	12.7

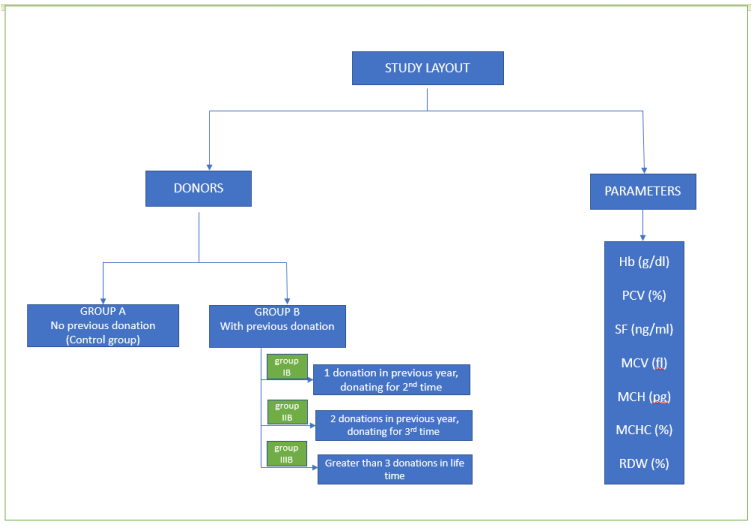

Figure 1: Study design.

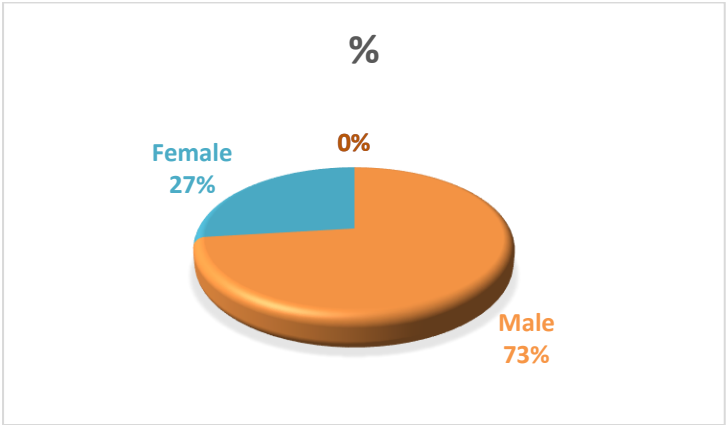

Figure 2: Percentage of Male and Female donors in the study

After taking informed consent of 234 donors, they were divided into 2 groups according to the study design shown in Figure 1: Group A and Group B. Group A (control group): (n- 133[56.84%]) comprised of blood donors who had not donated blood in previous years and were donating for the first time in which 80 were male donors and 53 were female donors. Group B: (n-102[43.4%]) comprised of repeat donors who had previously donated blood on one or more occasions and had 91 male donors and 10 female donors. Group B was further divided into 3 groups – group IB, IIB, and IIIB. Group IB: (n-53[22.65%]) comprised of donors who had donated once in the previous year and were donating for the second time. Out of total number of 53 donors, 46 were male and 7 were female. Group IIB: (n-17[7.26%]) 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 IIIB: (n-31[13.25%]) comprised of donors who had previously done 3 life time donations 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.Out of 234 donors, there were 171 male donors (73.08%) and 63 Female donors (27.92%) (Figure 2)On comparison of various hematological parameters between group A and group B male donors (Table: 1), 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 donors for parameters like hematocrit (PCV), MCV, MCH, and RDW. However, the MCHC values 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 Serum Ferritin values for first time male donors of group A (control group) and male donors of group B who were donating for more than first time was 80.08±62.65 ng/ml and 48.34±40.83 ng/ml respectively and this difference was found to be statistically significant (p= 0.0001).On comparison of various hematological parameters between group A and group B female donors (Table: 2), it was observed that none of the hematological parameters including mean Serum Ferritin values were found to be statistically significant. The mean of Serum

Ferritin values for first time female donors of group A and female donors of group B who were donating for more than first time was 39.58±31.83 ng/ml and 19.62±18.41 ng/ml respectively and this difference was found to be statistically insignificant (p value > 0.05). Inter-group calculation of mean of Serum Ferritin 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 (Table 4). We found a statistically significant correlation between frequency of donation (control groups and donating once or twice or thrice in a year) and decrease in serum ferritin levels. We found a statistically significant decrease in serum ferritin levels with the total number of donations in the life time (Table: 3).Among female donors the mean serum ferritin values showed a decreasing trend with increasing number of donations per year (Table 5). However, when inter-group comparison of mean of serum ferritin values was done for female donors it was not found to be statistically significant.Out of 234 donors, there were 35 donors (14.96%) who had serum ferritin <15 ng/ml. In 35 donors with low serum ferritin, 27 (15.79%) were male and 8 were female (12.70%) (Table 7). Distribution of donors on the basis of number of donations per year and serum ferritin <15 ng/ml was also seen in total number of donors within a group as well as for male and female donors separately (Table 6). The percentage of donors with low Serum Ferritin levels <15 ng/ml correlated with number of times blood donation was done, percentage was maximum in group IIB where donors had donated blood for two times previously in a year and were donating for the third time. The percentage of donors having Serum Ferritin <15 ng/ml was 47.06% in total number of donors, 43.75% in male donors. There was only one female donor in Group IIB and she had low Serum Ferritin < 15ng/ml (100%).

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 [7]. 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 [8]. Screening tests for potential blood donors require quicker, easier, and more cost-effective testing methods that do not require a venipuncture and cause minimal discomfort to the donor. The commonly used hemoglobin screening methods for blood donors are copper sulphate specific gravity method, Hemocue, cyanmethemoglobin, and Sahli's method. In the present study, pre-donation hemoglobin estimation was done using point of care portable Hemo Cue Hb photometer based on spectrophotometry. It is provided with a pack of microcuvettes containing reagents in dry form. The reaction in the microcuvette is a modified azide-methemoglobin reaction. Although the tests used for hemoglobin screening are quick, easy, and relatively inexpensive, their sensitivity, specificity, and accuracy are lower than that of an automated hematology analyzer [9]. So, the results were confirmed by running the EDTA venous sample of the donor on an automated analyzer. 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 non anemic donors and to ensure safe donor selection. The present study correlates well with the study done by Garg et al[2], Abdul Aziz et al[10], Jacob et al[11], Norashikin et al[12] ,Boulahriss et al [13] Nadarajan et al [15], and Milman et al [16]and. In all the studies including the present study there was statistically significant reduction in serum ferritin with an increase in the number of donations. However, Study done by Lim et al [14] showed different results, the mean of serum ferritin values did not fall significantly with recommended multiple donations but among male donors the mean serum ferritin values showed a decreasing trend with increasing number of donations per year. We found a statistically significant decrease in serum ferritin levels with the total number of donations in the life time (Table: 3) that correlated with the study done by Mahida VI et al [17]. Nevertheless, all the authors concluded that apart from hemoglobin estimation, estimation of serum ferritin should also be done to detect iron deficient non anemic state in repeat blood donors [13]. According to WHO Blood Safety, 2020 data; women constitute only 33% of blood donations globally which was also reflected in the present study. Percentage of female donors was much less as compared to male donors. Reasons for these gender difference could be prevalence of iron deficiency, pernicious anemia, a higher rate of adverse reactions to blood donation etc. which results in deferral of prospective female donors at the blood donation centers [18]. In the present study fall in serum ferritin values with increase in number of donations was not significant in female donors. This could be because only a small percentage of female donors participated. 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[19], Finch et al[20], Simon et al[21] and Abdul Aziz et al[10].Hematological parameters like hemoglobin, hematocrit, MCV, MCH, MCHC and RDW were also included in the present study. Observation of the results clearly indicates a greater fall of Serum Ferritin in group B male donors than group A male 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 in both genders. Such observations suggest routine hemoglobin values may not detect low levels of total iron store as portrayed by Serum Ferritin levelThe fall in hemoglobin and haematocrit mean values with the number of donation was not significant and this observation was consistent with the work of Abdul Aziz et al[10], Lim et al[14], Nadarajan et al[15], Garg et al[2]. MCV, MCH and RDW values reflected similar observations as by Jacob et al[11], Nadarajan et al[15], Lim et al[14] and Abdullah et al[6].

However, our observation of significant fall in MCHC level with donation did not correlate with the studies done by Jacob et al[11], Nadarajan et al[15], Garg et al[2] and Lim et al[14]. But this observation was within normal range of MCHC values. Out of 234 donors, there were 35 donors (14.96%) who had serum ferritin <15 ng/ml. This means that out of every 100 donors, approximately 15 donors had depleted iron stores and they were still donating blood because they were non anemic which has its own health implications. The percentage of donors with low Serum Ferritin levels <15 ng/ml correlated with number of times blood donation was done, percentage was maximum in group IIB (where donors had donated blood for two times previously in a year and were donating for the third time) in total number of donors as well as in male and female genders separately. This finding correlated with the study done by Deepa Devi G et al[22]. The percentage of donors having Serum Ferritin <15 ng/ml was 47.06% in total number of donors in group IIB, 43.75% in male donors of Group IIB. There was only one female donor in Group IIB and she had low Serum Ferritin < 15ng/ml (100%). These findings underscore the significance of doing Serum Ferritin in multiple time donors and so its utility in using it as an adjuvant to haemoglobin assessment in screening of non-anemic multiple time donors. In the present study, additional samples were taken in plain vacutainer after donation to analyze Serum Ferritin in all the blood donors included in the study. Then Serum Ferritin was analyzed using semiautomated ELISA analyzer, that requires turnaround time of about 4 hrs. In a practical setup, it won't be feasible to estimate serum ferritin for every donor as it has cost implications and waiting time. Also, Serum Ferritin is not an acute marker of iron deficiency, so it won't be fruitful to check for it before every donation. Still, it can be successfully employed to multiple time donors as we can see that percentage of donors with deficient iron stores are increasing with frequency of donation (Table 6) which becomes easier as they are more compliant compared to first time donors. Their contact details can be taken. Multiple time donors can be suggested to first get their serum ferritin levels checked. If it's within normal limits they can be called and informed that they can donate blood. Other way is to give iron supplementation once or twice a year and do serum ferritin once a year which would be more feasible and practical approach as we already know how much iron is lost with each donation. Remember donors donate blood with an intention to save some one's life. Nobody can repay them, but by taking care of their health, we can do the least. Also, it will maintain the donor pool. For this Serum ferritin can be of immense help.

CONCLUSION

The present study underscores the utility of Serum Ferritin level estimation as an adjuvant to hemoglobin assessment in multiple time donors. While there is clear cut consensus among blood banks to consider hemoglobin as a method for blood donor anemia screening, serum ferritin estimation can be planned before bleeding a prospective non anemic multiple time donor to correctly assess the iron status.

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