Short Report

Estimation of Serum Ferritin - A Better Screening Test for Blood Donors

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Abstract:

Background: Haemoglobin and haematocrit estimation are the commonest methods used worldwide in Blood Banks to screen donors. In order to detect Non-anaemic iron deficient state in repeat donors, these tests are inadequate. Several studies have reported a high incidence of iron deficiency in repeat donors.

Aims: The present study was undertaken to assess routine haematological parameters and body iron stores of blood donors in order to identify those who were potentially prone to develop iron deficiency anaemia.

Method: Predonation haemoglobin, haemogram and serum ferritin were done in 116 male donors. These were divided into two groups on the basis of number of donations.

Results: 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.

Conclusion: We 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 should be done in repeat donors. Iron supplementation for an adequate period post donation is recommended.

Keyword: Blood donors, blood donation, iron deficiency, haemogram, serum ferritin

Introduction:

Regular blood donation is a well-recognized entity amongst list of causes of iron deficiency.[1,2] Blood donation results in a substantial (200-250mg) loss of iron at each bleeding procedure (425-475ml) and therefore, the frequency of blood donation is so adjusted as to prevent anaemia in most donors.[3] However, the effect of blood donation on the status of total body iron content is often neglected, despite the fact that body iron reserve is small and therefore, iron depletion is better to be considered at least in blood donors and especially in repeat donors.[4,5] In the majority of blood banks, haemoglobin and/or haematocrit measurements are used as routine screening tests for allowing a person to donate blood.[1] This easy and inexpensive approach has been considered to be a reasonably good method of protecting donors against the development of progressive iron deficiency anaemia.[6] However, such routine methods do not reflect on the status of the total body iron content of an individual, an important marker to identify potentially anaemic subjects. Further, haemoglobin (Hb) measurement by Copper Sulphate (CuSO4) method is the routinely adopted method by majority of blood banks which lacks accuracy.[7] Even though the accepted normal cut off value is 12.5 gm%, a variation in Hb concentration of an individual due to physiological factors like plasma volume, red cell mass and altitude cannot be neglected for considering normal haemoglobin level.[8] It is well known that Hb does not decline until iron stores are completely exhausted and then only iron deficient erythropoiesis develops.[4] Thus it is reasonable and appropriate to seek for an alternative more appropriate parameter to look into the iron status of our repeat blood donors to identify potentially anaemic blood donors. 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.[9] 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.[10] Iron stores are classified as (a) depleted, when serum ferritin (SF) value is < 14 µg/l; (b) reduced, when value ranges between 15-30

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µg/l; (c) normal or replete, at value between 31-300 µg/l and (d) increased, keeping values beyond 300µg/l. Iron deficiency anaemia is considered present when SF is < 12 µg/l.[11] Published reports suggest very few studies have been conducted in this direction in India and none from the population of Uttarakhand state.

The present study was undertaken to estimate routine haematological parameters like Hb, haematocrit, red cell count, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red cell distribution width (RDW) and reticulocyte count for blood donors. In addition, a more logical and specific test which can identify the iron store status of the body like SF level in blood has also been included. The objective of the present study was to identify the blood donors who were potentially prone to develop anaemia by the SF level among the screened blood donors though their Hb level was within the normal range.

Materials and Methods:

A total of 116 male blood donors, who volunteered were recruited in the present study conducted at Blood Bank, Himalayan Institute of Medical Sciences, Dehradun. This study was cleared by the Institutional Ethics Committee. After taking informed consent, the subjects were divided into two groups: Group A (n-76) first time blood donors and Group B (n-40) repeat donors who had previously donated blood on one or more occasions (2nd blood donation n-26,

3rd n-3, 4th n-3, 5th n-2, 6th n- 4, 7th n-1, 21st n-1) and reported for the present donation. Donor registration form was filled and routine blood donor selection criteria were applied. Pre-donation Hb assessment was done using Hemocue method.[7] Blood donors having Hb in the range between 12.5 -16 gm/dl were included in the study. After blood donation of 350/450 ml of whole blood, additional samples were collected in plain and EDTA vacutainers and were processed within 4-6 hours of collection. EDTA blood was tested for complete haemogram using MS-9 haematology analyser. Reticulocyte percent i.e., number of reticulocyte per 100 RBC was done microscopically using New Methylene Blue stain.[12] For SF estimation, clear unhemolysed serum was separated from collected samples. The serum was stored in Eppendorf tubes at -20°C. SF was estimated by indirect solid phase enzyme immunometric assay (ELISA) using ELISA kit (ORGENTEC, Germany).

Statistical analysis was done using Microsoft Excel 2007. The data has been presented as Mean± SD. The statistical test used was Student's t test (unpaired). The value of pd"0.05 was taken as significant. Correlation between SF and number of donations and between Hb and SF was performed by Pearson correlation analysis.

Results:

All 116 (gr A, n-76; gr B, n-40) male blood donors were screened for haemogram and SF levels. All values have been presented as Mean ± SD with p values in Table-I.

Table - I

Comparison of haemogram and SF levels between two groups

Parameters	First time donors Gr A (n=76)	Repeat donors Gr B (n=40)	P-value
Age (y)	27.26±7.34	29.15±7.55	p=0.09
Haemoglobin (gm/dl)	14.15±0.93	14.14±0.91	p=0.49
Haematocrit (%)	41.97±2.80	41.96±3.45	p=0.98
MCH (pg)	31.35±2.94	30.46±3.53	p=0.14
MCV (fl)	92.93±8.42	89.87±7.87	p=0.05*
MCHC (g/dl)	33.75±1.60	33.86±2.07	p=0.95
RDW	13.66±1.05	13.89±1.42	p=0.32
RBC (x 10^6/mm^3)	4.54±0.45	4.70±0.58	p=0.05*
Reticulocyte %	0.46±0.30	0.57±0.36	p=0.04*
SF(ng/ml)	81.07±97.12	46.01±49.09	p=0.01*
SF<12 (ng/ml)	2.26±3.41 (n-8)	4.24±4.96 (n-11)	Iron deficiency anaemia
SF 12-14 (ng/ml)	13.25±0.65 (n-3)	12.19 (n-1)	Iron depleted
SF15-30 (ng/ml)	22.66±4.57 (n-18)	21.80±3.85 (n-10)	Iron reduced
SF31-300(ng/ml)	96.73±68.72 (n-43)	86.87±46.88(n-18)	Normal or iron replete
SF>300(ng/ml)	384.12±44.61 (n-4)	-	Increased iron

1: Data presented are mean±SD. P value <0.05 was taken as significant

The mean age of gr A and gr B was similar; the respective values were 27.26±7.34 and 29.15±7.55y. On comparison of various haematological parameters between gr A and gr B, it was observed that haemoglobin level in gr A was 14.15±0.93 and in gr B was 14.14±0.91gm/dl which was not statistically significant (pe"0.05). Further, no statistical significant difference was noted between two groups of donors for parameters like haematocrit, MCH, MCHC, and RDW. However, the red cell count (million/mm³ of blood) in gr A was 4.54±0.45 and in gr B was 4.70±0.58 and was statistically significant (p=0.05). The MCV values also showed a statistically significant difference (p=0.05) though in the normal range with gr A and gr B having values 92.93±8.42 and 89.87±7.87fl, respectively. Further, a significant difference (p=0.04) was observed in mean reticulocyte % in gr A and gr B having values 0.46±0.30 and 0.57±0.36 respectively indicating stimulated erythropoiesis in gr B. The SF level (ng/ml) in gr A was 81.07±97.12 and in gr B were 46.01±49.09

and was noted to be statistically significant (p= 0.01). A total of 23, gr A n- 11 (14.47%) and gr B n-12 (30%) subjects had shown SF level below the normal range (12-14ng/ml) but nobody had shown haemoglobin level below 12.5gm%. A total of 19, gr A n-8 (10.52%) and gr B n-11 (27.5%) had SF level <12ng/ml and showed iron deficient erythropoiesis. A scatter-gram Fig 1 was plotted to examine more critically for searching of a relationship between number of blood donations and SF level.

As depicted in the scatter-gram (Figure 1), a negative correlation was seen between the number of donations and SF level (r= -0.23).

It has been observed that there was a fall in SF value with the increase in number of blood donations, though the SF values of earlier donations were not known. It was further felt logical to see whether any relationship exists between the levels of SF and haemoglobin.
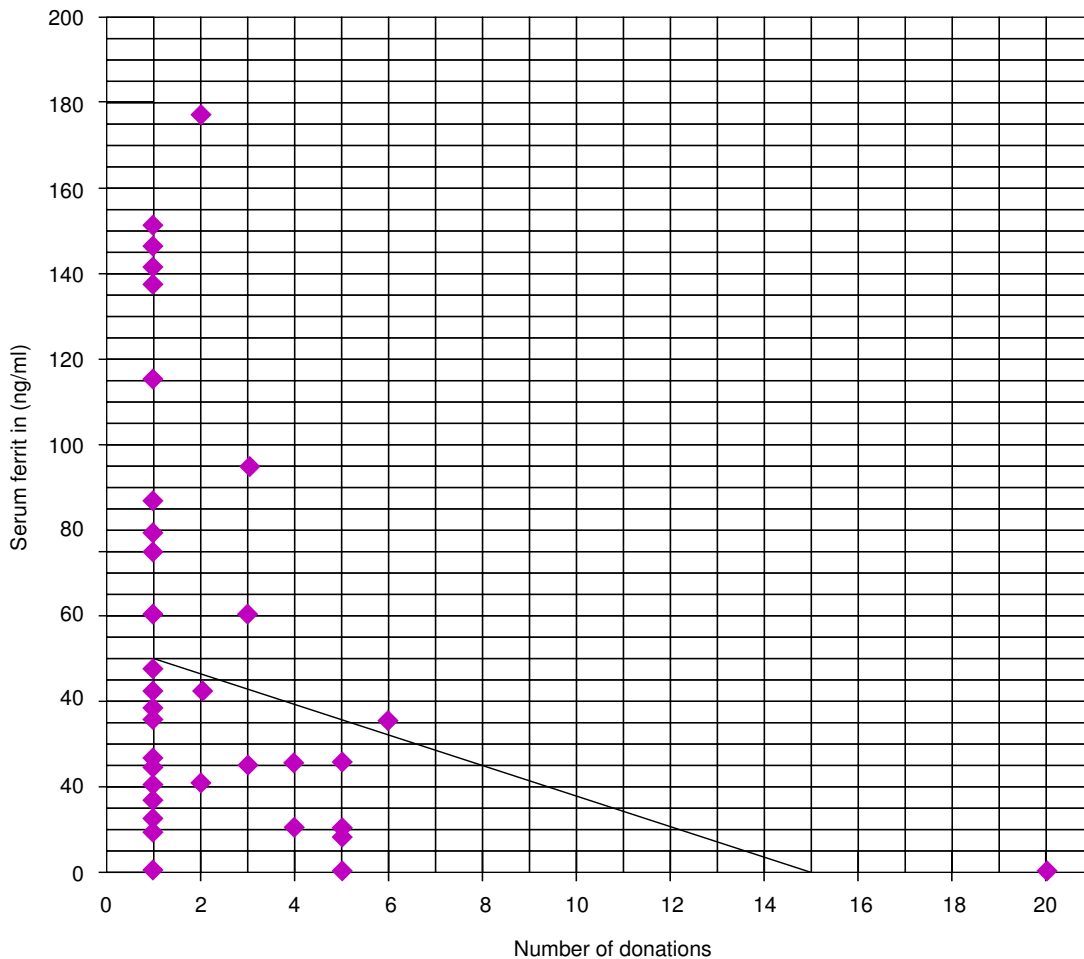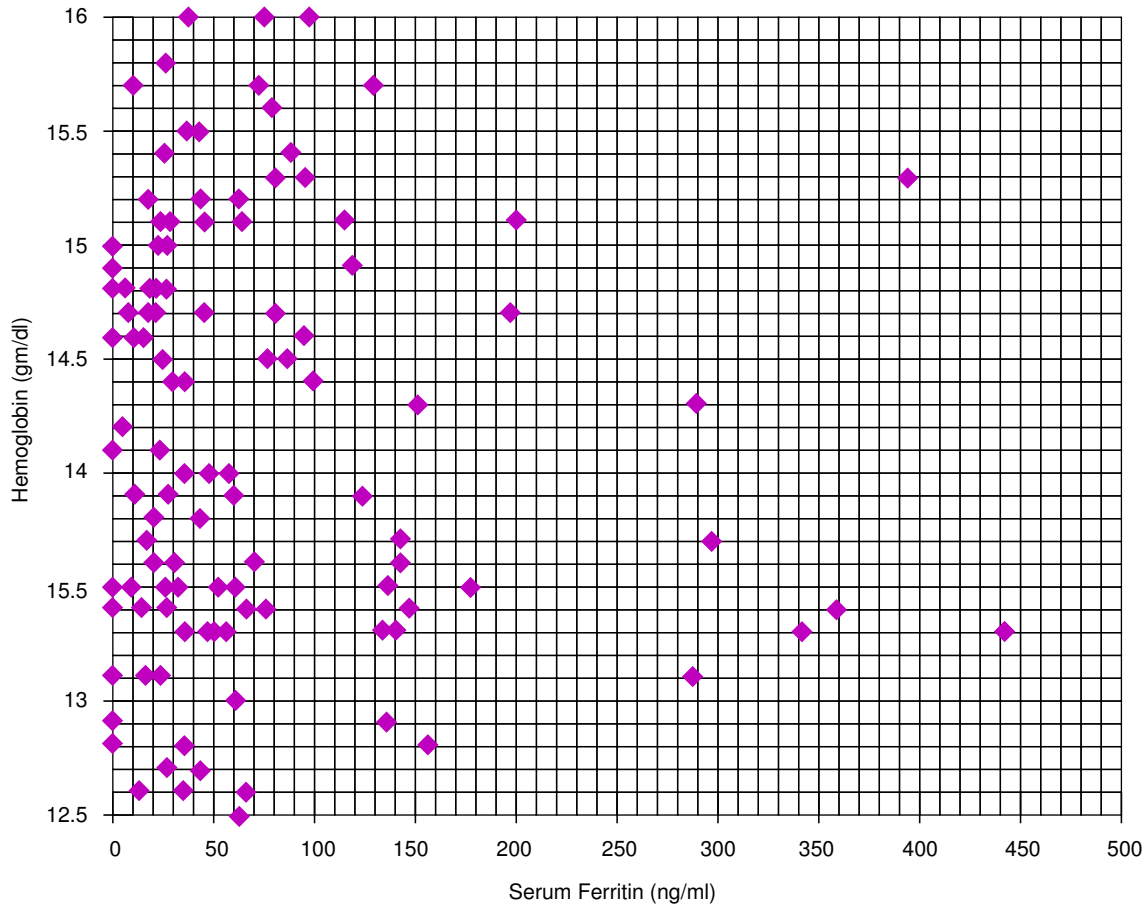

Fig.-1 : *Correlation between SF levels and number of donations*

Fig.-2 : *Correlation between Hb and SF levels*

When both values have been placed in scatter-gram (Figure 2) it was found that there was no correlation between these two observations (r= -0.06).

Discussion:

The results in Table-I indicate a greater fall of SF in gr B than gr A (p=0.01) though having similar mean values within normal range of haemoglobin, haematocrit, MCH, MCHC, RDW for identification of anaemia. Such findings suggested that the total iron store as portrayed by SF level, even when was at the lower level (gr A 14.47%, gr B 30%) could not be identified by the routine Hb values.[1] Further, a greater degree of fall in SF level, as observed in gr B (one donor who was donating on 21st occasion had shown SF value 0ng/ml, though haemoglobin level was 15gm/dl) could not be identified by the well-recognized cut off value of normal range of haemoglobin. Similar observations have been made by several researchers confirming the fact that Hb level estimation at the time of blood donation does not predict iron deficiency in a blood donor. A study from Chandigarh city, India also supported the same fact that Hb estimation alone is not sufficient to identify iron deficiency in any individual.[13] It is further important to mention that to replenish the iron store even in mild degree of anaemia needs the dietary care and specific treatment for at least three months, even if the Hb level is being corrected much earlier.[1,3,5,14-20] The lower the SF level, higher will be the possibility to suffer from anaemia i.e., potentially anaemic. Prevention of iron deficiency is essential as it is well known that anaemia causes non-specific symptoms like tiredness, weakness, headache, breathlessness, irritability, including the decreased work capacity.[17] Additionally, it is reported that iron depleted non anaemic females given iron supplements showed greater improvement in exercise performance as compared to unsupplemented controls.[18]

It was further interesting to note that the total RBC count was higher (p=0.05) in gr B than gr A. The repeat donors though having lower iron store, as predicted by SF level but had possibly compensated by increasing the total RBC count having relatively low MCV, thereby keeping the haemoglobin level normal which was corroborated by statistically significant (p=0.04) value of reticulocyte count in gr B . Such subjects are more prone to develop iron deficiency anaemia

and therefore, iron and vitamin C rich diet along with oral iron preparations should be prescribed to replenish the body iron store. The researchers from neighbouring Asian countries like Malaysia also reported the lower SF level in repeat donors and routinely advised the iron supplementation after each blood donation.[19,20] Study from Thailand and Iran have also concluded with recommendation of iron supplementation to repeat donors.[21,14]

A study from Gujarat comprised of vegetarian and non-vegetarian subjects but difference in SF levels was reported not significant because the amount of non-vegetarian food consumed was not adequate. Similar situation was also faced by our study as infrequent consumption of non-vegetarian food items was not able to much affect the SF levels.[22]

As compared to other studies, our study has included a complete haemogram with reticulocyte %. This has led to our understanding that in repeat donors, compensatory erythropoiesis takes place.

Conclusion

The present study therefore, indicated that SF level estimation in screened blood donors is essential to understand iron store status and may be introduced as a screening test before donating blood especially in repeat blood donor group of subjects even though their haemoglobin level is normal. Compensatory erythropoiesis takes place in repeat donors. Furthermore, Iron supplementation should be routinely advised to repeat blood donors.

Acknowledgements

Conflict of Interest : None

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