

ORIGINAL ARTICLE

Comparative Status of Iron Stores between Regular Repeat and First Time Voluntary Blood Donors: Prospective Study in Tertiary Care Hospital*Shailesh Kumar Mishra¹, Pawan Singh¹ and Nand Kishore Singh²**¹Department of Blood Transfusion, ²Department of Community Medicine, SHKM Govt. Medical College, Nalhar, Nuh, Haryana -122107, India.***Abstract:**

Background: Apart from challenging the bone marrow to increase its red cell production, thereby producing more blood for the donor, regular blood donation has been shown to have several benefits, one of which is preventing accumulation of body iron which can cause free radical formation in the body. The objective of the present study was to assess the comparative status of body iron stores between regular repeat and first time voluntary blood donors. **Material and Methods:** A total of 50 regular repeat (study) and 50 first-time (control) voluntary blood donors were studied prospectively. Ten millilitres of venous blood was drawn from each subject, 5 mL of which was put into sodium ethylenediamine tetra-acetic acid specimen bottles for a full blood count, including red blood cell indices. The remaining sample was allowed to clot in a plain container, and the serum was then retrieved for serum ferritin, serum iron, and serum transferrin receptor measurement by enzyme-linked immunosorbent assay. **Results:** Mean hemoglobin and packed cell volume in the study group (13.47 ± 2.36 g/dL and 42.00 ± 7.10 , respectively, $P = 0.303$) were not significantly higher than in the control group (12.98 ± 1.30 g/dL and 39.76 ± 4.41 , respectively, $P = 0.119$). Mean serum ferritin was 102.46 ± 80.26 ng/mL in the control group and 41.46 ± 40.33 ng/mL in the study group ($P = 0.001$). Mean serum ferritin for women in the study group (28.02 ± 25.00 ng/mL) was significantly lower than for women in the control group (56.35 ± 34.03 ng/mL, $P = 0.014$). Similarly, men in the study group had a lower mean serum ferritin (48.57 ± 45.17 ng/mL) than men in the control group (145.49 ± 87.74 ng/mL, $P = 0.00$). The mean serum transferrin receptor value was higher in the study group (1.56 ± 0.88 μ g/mL) than in the control group (1.19 ± 0.38 μ g/mL, $P = 0.033$). **Conclusion:** These findings suggest that hemoglobin concentration, packed cell volume, and serum iron levels are not significantly affected by regular blood donation and that regular blood donors appear

to have reduced iron stores compared with controls (first time blood donors).

Keywords: blood donors, hemoglobin, serum ferritin, serum iron, transferrin receptors

Introduction:

Regular blood donation has been found to be beneficial for many reasons. Apart from challenging the bone marrow such that hemopoietic activity is sustained and marrow remains red with continuous production of red cells, regular blood donation prevents accumulation of iron which can cause formation of free radicals in the body [1]. Important in the pathophysiology of diseases such as diabetes and cardiovascular disorders, is having iron available to participate in free radical reactions. This stems from the ease with which iron is reversibly oxidized and reduced. This property is essential for metabolic function, but makes iron potentially hazardous because of its ability to generate powerful oxidant species, such as hydroxyl radicals [2]. Iron plays a catalytic role in the Haber-Weiss reaction that leads to generation of superoxide anions, hydrogen peroxide, and hydroxyl radicals [2]. These oxidants can also trigger release of catalytic iron, initiating a vicious cycle that leads to formation of more reactive oxygen species [2]. In 1981, Sullivan first postulated the “iron hypothesis”, stating that chronic iron depletion has a protective effect against ischemic heart disease and may account for the lower risk of cardiovascular events in menstruating women [3, 4]. Because of its role in promoting lipid peroxidation, accelerated development of atherosclerosis has been postulated as a potential mechanism by which iron overload may increase the risk of ischemic cardiovascular events. In vitro studies

have shown that iron acutely promotes platelet reactivity and iron chelation inhibits activation of tissue factor [5, 6]. Elevated body iron stores were associated with an increased risk of myocardial infarction in a large cohort of Finnish men [7]. Iron may also have deleterious effects on vascular function by increasing reactive oxygen species locally, decreasing the bioavailability of nitric oxide, impairing vasorelaxation, and promoting platelet adhesion and aggregation [8]. Zheng et al provided evidence in support of the Sullivan hypothesis when they found that high frequency blood donors have decreased levels of serum ferritin (a marker of body iron stores), decreased levels of serum 3-nitrotyrosine (a marker of oxidative stress), and greater flow-mediated dilation in the brachial artery (a marker of vascular function) [9]. In another study, it was interesting to note that iron chelation with deferoxamine improved endothelial function in patients with coronary artery disease [10]. A Canadian study reported an increased risk of myocardial infarction in subjects from the highest serum iron category [11]. Another report by Kiechi et al from Italy found a positive association between serum ferritin levels and ultrasound measures of progression of carotid atherosclerosis over a five-year follow-up period [12]. The objective of the present study was to assess the comparative status of body iron stores between regular repeat and first time voluntary blood donors. The present study assessed body iron stores in regular repeat blood donors compared to first time blood donors. If an inverse relationship exists between regular donation and body iron stores, then regular blood donation may be advantageous in reducing total body iron stores and indirectly reducing the risk of cardiovascular events. This would indeed be a welcome addition to the many advantages of regular blood donation and could be used as a campaign tool to recruit potential blood donors and help to ameliorate the shortage of blood experienced in transfusion services, especially in our environment, where voluntary donation is for the most part, a myth, as well as enhancing the well-being of regular donors.

Material and Methods:

A total of 50 regular repeat (study) and 50 first-time (control) voluntary blood donors who came to donate blood in Blood Bank Shaheed Hasan Khan Mewati Government Medical College were studied. The study participants consisted of all blood donors over 18 years of age who had donated blood at least twice in the previous 12 months, four times in the previous 24 months, or at least six times in the previous 36 months, and gave their informed consent. Suitable donors donating for the first time were recruited as controls. Regular blood donors who refused to provide consent, pregnant and lactating women, and regular blood donors with a history of diabetes mellitus and cardiovascular disease were excluded from participation in the study.

The sample size was calculated by using formula.

Blood was collected after recording of the relevant information, including age, gender, history of blood donation, and history of hypertension and/or diabetes mellitus, in a questionnaire. First, 10 mL of venous blood was drawn from each subject, of which 5 mL was put into a sodium ethylene diamine tetra-acetic acid (EDTA) specimen bottle for a full blood count, including red blood cell indices, using a KX 21 N hematology analyzer (Sysmex Corporation, Kobe, Japan). This was analyzed within two hours of collection. The remaining 5 mL of blood was transferred to a plain screw-capped disposable plastic tube and allowed to stand at room temperature for about two hours until clotted, at which time the clot was retracted. This was then centrifuged, and the serum was then separated, transferred to plain cryotubes using a transfer pipette, and stored at -72°C until analysis. This was used to measure serum ferritin, serum iron, and serum transferrin receptor levels. Serum ferritin and serum transferrin receptor levels were determined by enzyme linked immunosorbent assay. A commercial ferritin assay kit manufactured by Biotech Clinical Laboratories Inc (Novi, MI, USA) was used to determine serum ferritin, a commercial human enzyme-linked immunoassay kit (MyBioSource, San Diego, CA, USA) was used to measure serum transferrin receptor levels, and a commercial iron assay kit (Roche

Diagnostics GmbH, Mannheim, Germany) was used to determine serum iron.

The data were analyzed using Statistical Package for the Social Sciences for Windows version 11.5 software (SPSS Inc, Chicago, IL, USA) and Microsoft Excel. Descriptive statistics, the χ^2 test, and the Student's t-test were used as appropriate. The critical level of statistical significance was set at P, 0.05.

Results:

Demographic characteristics - A total of 100 subjects aged above 18 to 65 years were enrolled, of whom 50 were regular repeat blood donors (study group) and 50 were first-time donors (control group). The mean age was 35.19 ± 7.94 years in the study group and 33.77 ± 9.15 years in the control group ($P = 0.461$). There were 42 men and 8 women in the study group and 46 men and 4 women in the control group. Table 1 shows the mean hemoglobin, packed cell volume, serum iron, mean serum ferritin, and mean serum transferrin receptor values according to gender. As expected, men had higher mean hemoglobin and packed cell volume (13.80 ± 1.69 g/dL, $42.57\% \pm 5.61\%$) than women (12.48 ± 2.30 g/dL, $39.00\% \pm 6.66\%$) and the difference was statistically significant ($P = 0.004$ and $P = 0.011$, respectively). There were no significant differences in the means for red cell indices between men and women. Mean serum iron in the men (12.26 ± 4.20 $\mu\text{m/L}$) was higher than in women (10.34 ± 3.64 $\mu\text{m/L}$) and this difference was statistically significant ($P = 0.042$). Mean serum ferritin levels were also significantly higher in men (78.24 ± 75.40 ng/mL) than in women (40.41 ± 32.13 ng/mL, $P = 0.009$). Comparison of mean serum transferrin receptor levels showed no difference between the genders (1.39 ± 0.52 $\mu\text{g/mL}$ in men and 1.47 ± 1.04 $\mu\text{g/mL}$ in women, $P = 0.650$). A comparison of mean hemoglobin, packed cell volume, mean serum iron, mean serum ferritin, and mean serum transferrin receptor levels in the study and control groups is shown in Table 2. Mean hemoglobin in the study group (13.47 ± 2.36 g/dL) was not significantly higher than in the control group (12.98 ± 1.30 g/dL, $P = 0.303$). Similarly, even though the mean packed cell volume for the study group

($42.00\% \pm 7.10\%$) was higher than in the control group ($39.76\% \pm 4.14\%$), the difference did not reach statistical significance ($P = 0.119$). Mean cell volume was significantly lower in the study group (80.49 ± 7.44 fl) than in the control group (85.49 ± 6.25 fl, $P = 0.003$), but mean serum iron in the study group (11.58 ± 4.53 $\mu\text{m/L}$) was not different from that in the control group (11.44 ± 3.18 $\mu\text{m/L}$, $P = 0.884$). However, mean serum ferritin in the control group (102.46 ± 80.26 ng/mL) was higher than in the study group (41.46 ± 40.33 ng/mL) and this difference was statistically significant ($P = 0.001$). The mean serum transferrin receptor value was also significantly higher in the study group (1.56 ± 0.88 $\mu\text{g/mL}$) than in the control group (1.19 ± 0.41 $\mu\text{g/mL}$, $P = 0.033$).

Table 3 shows a comparison of mean serum iron, ferritin, and transferrin receptor levels according to gender in the study group and the control group. In both groups, women had a lower mean serum iron but this was not statistically significant. However, in the study group, women had significantly lower mean serum ferritin levels (28.02 ± 25.00 ng/mL) than men (48.57 ± 45.17 ng/mL, $P = 0.040$). Similarly, in the control group, women had significantly lower mean serum ferritin levels (56.35 ± 34.03 ng/mL) than men (145.49 ± 87.74 ng/mL, $P = 0.001$). Women in the study group also had significantly lower serum ferritin levels (28.02 ± 25.00 ng/mL) than women in the control group (56.35 ± 34.03 ng/mL, $P = 0.014$). Similarly, men in the study group had significantly lower mean ferritin levels (48.57 ± 45.17 ng/mL) than men in the control group (145.49 ± 87.74 ng/mL, $P = 0.000$). The mean serum transferrin receptor level in the control group was also expectedly higher in women (1.22 ± 0.44 $\mu\text{g/mL}$) than in men (1.15 ± 0.39 $\mu\text{g/mL}$). However, this difference did not reach statistical significance ($P = 0.650$). In the study group, women did not have significantly higher mean serum transferrin receptor levels (1.66 ± 1.32 $\mu\text{g/mL}$) compared with men (1.50 ± 0.55 $\mu\text{g/mL}$, $P = 0.625$). Women in the study group also had a higher mean serum transferrin receptor level (1.66 ± 1.32 $\mu\text{g/mL}$) compared with women in the control group (1.22 ± 0.44 $\mu\text{g/mL}$, $P = 0.196$), but again the difference was not statistically

significant. However, men in the study group had a significantly higher mean serum transferrin receptor level ($1.50 \pm 0.55 \mu\text{g/mL}$) compared with men in the study group ($1.15 \pm 0.39 \mu\text{g/mL}$, $P = 0.013$).

Table No.1: Mean hemoglobin concentration, packed cell volume, red cell indices, serum iron, serum ferritin, and serum transferrin receptors according to gender

	Women (n = 12)	Men (n = 88)	Total (n = 100)	P value
Hb (g/dL)	12.48 ± 2.30	13.80 ± 1.69	13.29 ± 2.04	0.004
PCV (%)	39.00 ± 6.66	42.57 ± 5.61	41.18 ± 6.25	0.011
MCV (fl)	82.35 ± 8.04	82.30 ± 7.04	82.32 ± 7.39	0.977
MCH (pg)	27.08 ± 3.25	28.53 ± 8.36	27.97 ± 6.84	0.351
MCHC (g/dL)	32.13 ± 1.82	32.32 ± 2.00	32.25 ± 1.92	0.658
Serum iron ($\mu\text{m/L}$)	10.34 ± 3.64	12.26 ± 4.20	11.53 ± 4.08	0.042
Serum ferritin (ng/mL)	40.41 ± 32.13	78.24 ± 75.40	63.30 ± 64.48	0.009
sTfr ($\mu\text{g/mL}$)	1.47 ± 1.04	1.39 ± 0.52	1.42 ± 0.76	0.650

Abbreviations: Hb, hemoglobin; sTfr, serum transferrin receptor; PCV, packed cell volume; MCV, mean cell volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration.

Table No. 2: Mean hemoglobin concentration, packed cell volume, red cell indices, serum iron, serum ferritin, and serum transferrin receptor levels in the study and control groups

	Study group (Regular repeat donor) n = 50	Control group (First time donor) n = 50	P value
Hb (g/dL)	13.47 ± 2.36	12.98 ± 1.30	0.303
PCV (%)	42.00 ± 7.10	39.76 ± 4.14	0.119
MCV (fl)	80.49 ± 7.44	85.49 ± 6.25	0.003
MCH (pg)	27.78 ± 8.42	28.30 ± 2.42	0.743
MCHC (g/dL)	31.91 ± 2.19	32.83 ± 1.15	0.036
Serum iron ($\mu\text{m/L}$)	11.58 ± 4.53	11.44 ± 3.18	0.884
Serum ferritin (ng/mL)	41.46 ± 40.33	102.46 ± 80.26	0.001
sTfr ($\mu\text{g/mL}$)	1.56 ± 0.88	1.19 ± 0.41	0.033

Abbreviations: Hb, hemoglobin; sTfr, serum transferrin receptor; PCV, packed cell

Table No.3: Mean serum iron, ferritin, and serum transferrin receptors (sTfr) in study and control groups segregated according to gender

	Serum iron in $\mu\text{m/L}$	Serum ferritin in ng/mL	serum transferrin receptor (sTfr) in $\mu\text{g/mL}$
Study group (Regular repeat donor)			
Females (n = 8)	10.41 \pm 4.15	28.02 \pm 25.00	1.66 \pm 1.32
Males (n = 42)	12.16 \pm 4.65	48.57 \pm 45.17	1.50 \pm 0.55
Total (n = 50)	11.58 \pm 4.53	41.46 \pm 40.33	1.56 \pm 0.88
Control group(First time donor)			
Females (n = 4)	10.25 \pm 3.00	56.35 \pm 34.03	1.22 \pm 0.44
Males (n = 46)	12.47 \pm 3.06	145.49 \pm 87.74	1.15 \pm 0.39
Total (n = 50)	11.44 \pm 3.18	102.46 \pm 80.26	1.19 \pm 0.4
P values			
Study women versus study men	0.171	0.040	0.625
Control women versus control men	0.055	0.000	0.650
Study women versus control women	0.900	0.014	0.196
Study men versus control men	0.780	0.001	0.013

Discussion:

Blood banks have a responsibility to protect blood donors and prevent them against developing anemia. They also have an important role to play in encouraging voluntary blood donation in a society like South Haryana with majority of muslim population where blood donation is viewed with skepticism. In this study, there was no significant difference in hemoglobin concentration between regular repeat blood donors and first time donors ($P = 0.303$ and $P = 0.119$, respectively). This is similar to the observations of Flesland et al, who reported that the hemoglobin concentration in regular blood donors was not significantly different from that in first-time donors [13]. Similarly, Szymczy-Nuzka and Woloweic reported a normal hemoglobin and packed cell volume in 151 regular male donors who had given over ten units of whole blood at a frequency of 3-4 units per year [14]. Denicourt and Goudemand also found no significant difference in hemoglobin and packed cell

volume in 394 women who had given three donations yearly for 2–10 years, compared with new donors [15]. Further, several studies have reported no cases of anemia in male and female donors donating 1800–1400 mL of blood annually [16,17], but this finding is in contrast with the work of Djalali et al [18], as well as that of Zaccheus and Baribefe [19], who reported a significantly lower hemoglobin and packed cell volume in regular blood donors when compared with healthy controls. In our study, the mean serum ferritin level was significantly lower in the study group than in the control group ($P = 0.001$). Szymczyk-Nuzka and Woloweic, when determining the prevalence of iron deficiency in regular blood donors, noted a lower serum ferritin level in this group in contrast with first-time donors who had normal serum ferritin levels [14]. Norashikin et al also reported significantly lower serum ferritin levels in regular blood donors [20]. However, Vilzu et al found no significant difference

between ferritin levels in controls and donors donating less than 20 units [21].

Several researchers have noted a significant reduction in ferritin levels with increasing donations [19-25]. Even though we reported a lower serum ferritin level with increasing number of donations, this pattern failed to reach statistical significance, which may reflect the small sample size or be due to increased iron absorption subsequent to a compensatory increase in hemopoiesis in the face of tightly regulated loss. The lack of a statistically significant difference in mean serum iron between the study and control groups is consistent with the results of a study by Akpotuzor et al who concluded that there was no observable difference in biochemical iron parameters between regular donors and healthy controls [26]. In contrast, several other studies have documented lower serum iron levels in regular blood donors compared with healthy controls [19]. In order to determine the usefulness of serum iron and ferritin for accurate diagnosis of iron deficiency in hospital patients, Burns et al compared the results of serum iron and ferritin

with the presence of stainable iron in bone marrow aspirates from 301 patients [27]. Using standard criteria, iron deficiency was correctly diagnosed by serum iron in only 41% and ferritin in 90% of patients, respectively. These investigators concluded that iron measurements lack the prerequisite sensitivity and/or specificity for accurate diagnosis of iron deficiency. The various other studies done recently on this topic also collaborates the finding of present study [28-31].

Conclusion:

Our data suggest that hemoglobin concentration, packed cell volume, and serum iron levels are not significantly affected by regular repeat blood donation. Regular repeat blood donors appear to have reduced iron stores when compared with first time blood donor, as shown by significantly reduced serum ferritin levels and raised serum transferrin receptor levels.

Conflict of Interest - Nil

Sources of Support - Nil

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