Table 6. Comparison for hematological and biochemical parameters in different groups as per frequency of donation.

Parameters	Mean ± Standard Deviation				p-value
	Group A (1-5 times) (n=151)	Group B (6-10 times) (n=48)	Group C (11-15 times) (n=27)	Group D (>15 times) (n=62)	
Hb (gm/dL)	14.13±0.3	14.01±22	14.4±1.49	14.2±1.78	<0.0001
MCV (fL)	88.37±3.9	86.29±5.8	90.37±6.73	87.03±8.8	<0.0001
MCH (pg)	28.5±1	27.42±1	29.6±2.75	27.5±3.5	<0.0001
MCHC (g/dL)	32.14±0.3	31.68± 29.2	32.6±1.2	31.5±1.26	<0.0001
S. Iron (µg/dL)	113.4± 32.88	116.47± 25.6	127.2±34.2	125.3± 25.6	<0.0001
S. Ferritin (ng/mL)	48.17± 23.75	47.64± 59.2	52.62±24.7	39.09± 15.5	<0.0001
TIBC (mg/dL)	363.39± 9.8	397.7± 54.9	391.55±53.6	427.02± 52.1	<0.0001

Table 7. Comparison for hematological and biochemical parameters in different groups as per last donation interval.

Parameters	Mean ± Standard Deviation				p-value
	Group 1 (3 months) (n=61)	Group 2 (>3-6 months) (n=101)	Group 3 (>6-9 months) (n=18)	Group 4 (>9 months) (n=108)	
Hb (gm/dL)	14.28± 1.4	13.9±22	14.3± 1.4	14.3± 1.4	<0.0001
MCV (fL)	86.02± 5.8	88.83± 5.8	88.25± 6.7	88.11± 6.7	<0.0001
MCH (pg)	27.02± 2.3	28.6±0	28.3± 2.7	28.47± 2.7	<0.0001
MCHC (g/dL)	31.34± 1.1	32.1±29.2	32.05± 1.4	32.1±1.4	<0.0001
S. Iron (µg/dL)	132.45± 33.3	106.08± 25.8	113.66± 34.2	121.19± 26.8	<0.0001
S. Ferritin (ng/mL)	37.6±31.8	37.07±59.5	48.85± 24.7	60.07± 34.1	<0.0001
TIBC (mg/dL)	424.31± 50.1	383.49± 55.1	396.52± 53.6	363.51± 56.1	<0.0001

Table 8. Comparison for hematological and biochemical parameters in different groups as per age distribution of blood donors.

Parameters	Mean ± Standard Deviation				p-value
	Group I (≤20 yrs.) (n=40)	Group II (21-30 yrs.) (n=120)	Group III (31-40 yrs.) (n=62)	Group IV (> 40 yrs.) (n=66)	
Hb (gm/dL)	13.6±1.3	14.3±0.49	14.2±20	14.16±22	<0.0001
MCV (fL)	88.54±5.0	87.58±4.9	88.11±4.1	88.01±5.85	<0.0001
MCH (pg)	28.49±2.1	28.2±1.55	28.35±2.1	27.92±2.11	<0.0001
MCHC (g/dL)	32.07±1.1	32.08±0.2	32.08± 21.1	31.61±29.2	<0.0001
S. Iron (µg/dL)	99.59± 33.9	117.03± 27.7	125.08± 26.4	123.42± 25.6	<0.0001
S. Ferritin (ng/mL)	35.07± 29.6	44.7±19.8	57.1±52.3	46.7± 59.2	<0.0001
TIBC (mg/dL)	356.21± 63.8	379.45± 15.5	396.91± 67.2	403.3± 54.9	<0.0001

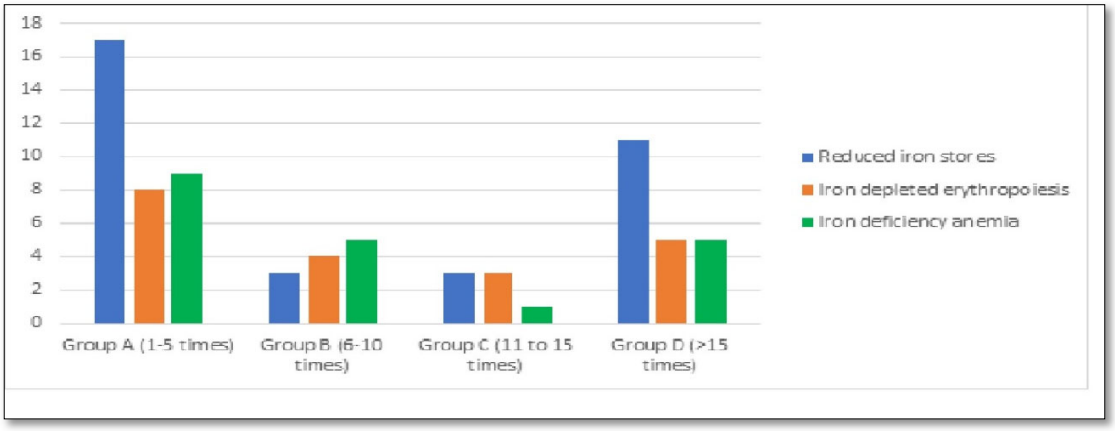

Figure 1. Iron deficiency stages in different groups as per frequency of donation.

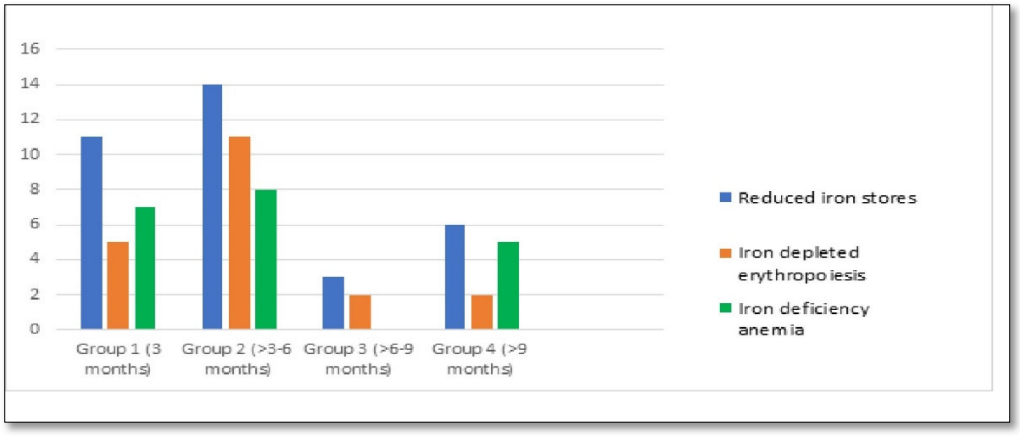

Figure 2. Iron deficiency stages in different groups as per last donation interval.

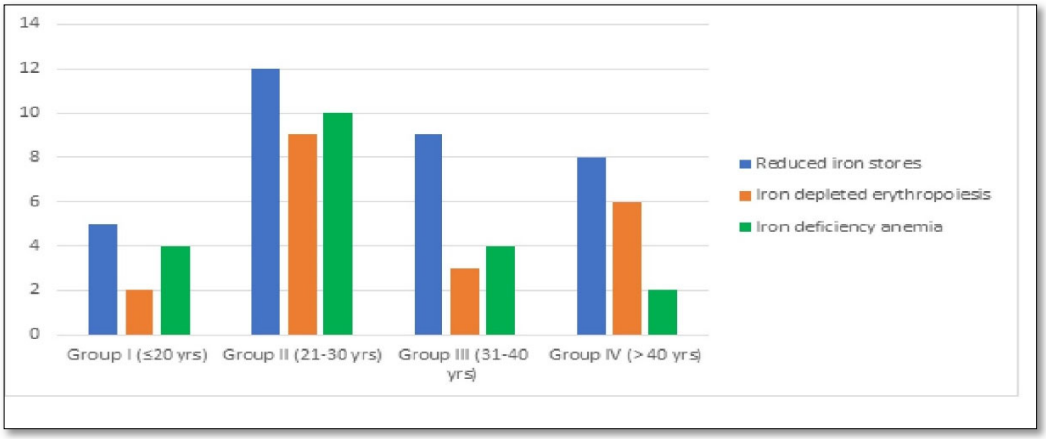

Figure 3. Iron deficiency stages in different groups as per age distribution of blood donors.

DISCUSSION

The adequate and quality supply of blood depends on the health status of blood donors. Although, repeated regular blood donation is considered a good donation and in order to fulfill the necessities, blood centers find it more effective to collect blood from existing donors than to recruit new donors but this strategy leads to iron depletion of these donors [5]. In our study, the Hb level was above the normal cut off value for blood donation in all the blood donors, including the ones who were later found to have reduced iron stores. In our study, the mean Hb level in all the donors was 14.178 g/dL. However, the Hb levels varied significantly among the donors depending on the duration of last donation. All our results

have been compared with the data from other published articles from India and abroad. It is clear from the **Table 6** that the donors of Group C have higher levels of Hb, MCV, MCH, MCHC, S. Iron and S. Ferritin as compared to donors of other groups. The mean value of S. Iron and TIBC increased as the frequency of donation increased. The highest levels with respect to all the biochemical parameters except TIBC are seen in donors of Group C followed by donors of Group A and Group D having lowest level of S. Iron and S. Ferritin respectively. TIBC is seen to show inverse relation with frequency of donation. The variation of parameters among the group is statistically significant as the p value is less than 0.0001. The data presented in the **Table 7** shows the mean Hb, MCV, MCH, MCHC, S. Iron, S. Ferritin, and TIBC in different groups as per last donation interval. It is clear from **Table 7** that the donors of Group 4 have higher levels of Hb, MCV, MCH and S. Ferritin as compared to donors of other groups. The variation of parameters among the group is statistically significant as the p value is less than 0.0001. The data presented in the **Table 8** shows the mean Hb, MCV, MCH, MCHC, S. Iron, S. Ferritin, and TIBC in different groups as per age distribution of the blood donors. It is clear from **Table 8** that the donors of Group III have higher levels of S. Iron and S. Ferritin as compared to donors of other groups. The mean value of S. Iron, S. Ferritin and TIBC increased as the age distribution of donors increased. The highest levels with respect to all the biochemical parameters except TIBC are seen in donors of Group III followed by donors of Group IV and Group II, and Group I having lowest level of S. Iron and Ferritin respectively. The variation of parameters among the group is statistically significant as the p value is less than 0.0001. There is a significant relation of hematological parameters including MCV, MCH with increased frequency of donation, consistent with other study from North India [6] in which MCV, MCH dropped significantly in relation to annual donation frequency. Among the biochemical tests for iron stores, S. Ferritin is considered as a reliable indicator of iron status in blood donors. S. Ferritin alone or in combination with other tests are helpful in assessing the iron status of the blood donors [7]. In the present study, mean S. Ferritin concentration of 46.54 ng/mL was recorded amongst donors. As clearly seen from **Figure 4**, the mean value of S. Ferritin dropped significantly in donors as the frequency of donation increased proving the influence of the number of donations on serum ferritin levels. The result of our study showed 34 (11.8%) of repeat blood donors had reduced iron stores (S. Ferritin 15-20 ng/mL), 20 (6.94%) of repeated blood donors had IDE (S. Ferritin 12-15 ng/mL) and 20 (6.94%) of the donors had iron deficiency anemia (S. Ferritin <12 ng/mL). Analysis of the data presented in **Table 3** and **Figure 1** shows the iron deficiency stages in different donor groups as per frequency of donation. From the data obtained it is evident that amongst all four groups, maximum number of donors, who had reduced iron stores (17), iron depleted erythropoiesis (8) and iron deficiency anemia (9) belong to Group A followed by donors of Group D where donors had reduced iron stores (11), iron depleted erythropoiesis (5) and iron deficiency anemia (5). Iron deficient erythropoiesis was seen in donors who donated blood 1-5 times (n= 17) and was more than in donors who donated more than 15 times (n=62). Analysis of the data presented in **Table 4** and **Figure 2** shows the iron deficiency stages in different donor groups as per last donation interval. From the data obtained it is evident that amongst all four groups, maximum number of donors, who had reduced iron stores (14), iron depleted erythropoiesis (11) and iron deficiency anemia (8) belong to Group 2 followed by donors of Group 1 where donors had reduced iron stores (11), iron depleted erythropoiesis (5) and iron deficiency anemia (7). Analysis of the data presented in **Table 5** and **Figure 3** shows the iron deficiency stages in different donor groups as per age distribution of blood donors. From the data obtained it is evident that amongst all four groups, maximum number of donors, who had reduced iron stores (12), iron depleted erythropoiesis (9) and iron deficiency anemia (10) belong to Group II followed by donors of Group III and IV where donors had reduced iron stores (9; 8), iron depleted erythropoiesis (3; 6) and iron deficiency anemia (4; 2). Iron depletion is the earliest stage of iron deficiency, and signifies that iron stores are decreased or absent, but the serum iron concentration and blood hemoglobin levels are normal. Iron deficiency without anemia is somewhat more advanced stage of iron deficiency, characterized by decreased or absent iron storage, usually a low serum iron concentration and low blood hemoglobin concentration, but without anemia [8]. Our study showed direct relationship of serum iron, ferritin with number of blood donation and direct relation with TIBC levels i.e., TIBC levels are increased with increasing donation number. There was a significant correlation between inter-donation and last donation interval with serum ferritin levels. This finding was consistent with previous studies [9,10] in which the S. Ferritin levels were significantly lower in regular donors and female donors. In a similar study, reviewing of screening criteria used at the time of donation and inclusion of S. Ferritin measurement in the assessment of regular blood donors to secure adequate iron reserves in the donor population was recommended [11]. A study recommends measurement of S. Ferritin levels after five donations to help identify iron deficient individuals [12]. In a study, it was concluded that the introduction of routine S. Ferritin analysis resulted in an increase of mean Hb levels in blood donors particularly in women of childbearing age. The incidence of pre donation anemia and donation ineligibility due to a low Hb concentration decreased significantly. The return intervals of donors rejected on account of low Hb levels were also shortened. Therefore, early detection of iron deficiency and anemia among blood donors would allow appropriate re-adjustment of donation intervals and would guide the use of iron supplementation to prevent the development of iron deficiency anemia in the donors thereby preventing shrinking of donor pool [13]. In fact, a study demonstrated that only 25% of first-time donors return to the blood donation facility

after rejection, while 47% of first-time donors come back within 6 months when accepted at their first visit [14]. One promising approach involves administration of carbonyl iron rather than ferrous sulfate, a formulation that is well tolerated by most individuals [15].

CONCLUSION

According to the results obtained from our study, a total of 74 (25.69%) repeat voluntary blood donors were found to be having depleted iron stores and thus, it can be concluded that as the blood donation frequency increases, the S. Ferritin and S. Iron decreases significantly. Hb measurement alone is not enough to evaluate donor suitability, especially in regular blood donors. Also, shorter the inter donation interval, greater the chances of iron depletion. Current guidelines for blood donation i.e., Hb levels ≥ 12.5 g/dL does not reflect true iron status of the blood donor. Therefore, more sensitive tests like S. Iron and S. Ferritin in blood donors are important markers. Donors need to be educated about iron deficiency and recommended iron rich diet which can be easily available. Those who have donated more than twice should be put on regular iron supplementation to prevent the iron store depletion. In fact, a policy for investigation and management of iron deficiency anemia needs to be incorporated as a regular routine procedure in blood transfusion services. The results provide an insight into the importance of monitoring the iron status of regular repeat donors, thereby, ensuring best of health care to donors and maintaining a regular pool of donors. Now, that the guidelines lay more stress and emphasis on regular repeated voluntary non-remunerated blood donations, our criteria for donor pre-donation testing needs to be revisited.

REFERENCES

1. Amilo GI, Ifeanyichukwu MO, Ngwu AM, Obi GO (2014) Iron deficiency in regular blood donors in Enugu (Southeastern Nigeria). Int J Blood Transfus Immunohematol 4: 1-6.
2. Nadarajan VS, Eow GI (2002) Anemia and iron status among blood donors in a blood transfusion unit in Malaysia. Malays J Pathol 24(2): 99-102.
3. Suominen P, Punnonen K, Rajamäki A, Irjala K (1998) Serum transferrin receptor and transferrin receptor-ferritin index identify healthy subjects with subclinical iron deficits. Blood 92(8): 2934-2939.
4. Jeremiah ZA, Koate BB (2010) Anemia, iron deficiency and iron deficiency anemia among blood donors in Port Harcourt, Nigeria. Blood Transfus 8(2): 113-117.
5. Popovsky MA (2012) Anemia, iron depletion, and the blood donor: It's time to work on the donor's behalf. Transfusion 52: 688-692.
6. Dara RC, Marwaha N, Khetan D, Patidar GK (2016) A Randomized Control Study to Evaluate Effects of Short-term Oral Iron Supplementation in Regular Voluntary Blood Donors. Indian J Hematol Blood Transfus 32(3): 299-306.
7. Kiss JE, Brambilla D, Glynn SA, Mast AE, Spencer BR, et al. (2015) Oral iron supplementation after blood donation: A randomized clinical trial. JAMA 313(6): 575-583.
8. Alharbi SH, Alateeq FA, Ahmed IB, Alsogair ARAA, Al-Rashdi YDA, et al. (2018) Assessment of levels of awareness towards blood donation in Saudi Arabia. AIMS Public Health 5(3): 324-337.
9. Moghadam AM, Natanzi MM, Djalali M, Saedisomeolia A, Javanbakht MH, et al. (2013) Relationship between blood donor's iron status and their age, body mass index and donation frequency. Sao Paulo Med J 131(6): 377-383.
10. Ali BH, Osaro E, Sani I, Wase A, Festus O, et al. (2015) Prevalence of iron deficiency anemia among blood donors in Sokoto, North Western, Nigeria. J Coast Life Med 3(4): 312-316.
11. Tailor HM, Patel PR, Pandya AKNP, Mangukiya S (2017) Study of Various Hematological Parameters and Iron Status among Voluntary Blood Donors Int J Med Public Health 7(1): 61-65.
12. Alvarez-Ossorio L, Kirchner H, Klüter H, Schlenke P (2000) Low ferritin levels indicate the need for iron supplementation: Strategy to minimize iron-depletion in regular blood donors. Transfus Med 10: 107-112.
13. O'Meara A, Infanti L, Stebler C, Ruesch M, Sigle JP, et al. (2011) The value of routine ferritin measurement in blood donors. Transfusion 51(10): 2183-2188.
14. Boulton F (2008) Evidence-based criteria for the care and selection of blood donors, with some comments on the relationship to blood supply, and emphasis on the management of donation-induced iron depletion. Transfus Med 18(1): 13-27.
15. Reiss RF, Kessler D (2010) Current concerns for blood donor well-being and health. South Med J 103(4): 343-346.