

# Impact of Regular Blood Donation on Body Iron Stores at Saudi Blood Donors

Mohammed Qassadi, Saleh Mohammed Abdella, Taqwa Youssife

College of Nursing and Health Sciences, Jazan University, Jazan, Kingdom of Saudi Arabia

Email: Modatheeb@gmail.com

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## Abstract

**Introduction:** One of the most frequent observations in long-term blood donation is chronic iron deficiency, which can develop into anaemia. The majority of blood screening methods employed by blood banks do not incorporate iron-status markers, which may result in potential subclinical iron deficiency. The aim of this study was to evaluate the effects of repeated blood donation on the levels of iron in the body and to guide blood donors in preventing the depletion of iron stores. **Methods:** Regular blood donors were categorised into distinct groups according to the number of donations they gave, and then the correlation between these groups and their bodies' iron levels was examined. Different parameters were employed to identify iron deficiency and iron depletion in blood donors: serum ferritin, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), total iron-binding capacity (TIBC), and serum iron. **Results:** The study included 300 individuals who regularly and willingly donated blood. There were no iron insufficiency cases among those donating blood for the first time (Group I). However, 15.5% of individuals who had donated once before (Group II) had ferritin levels of 15 - 30 µg/dl (ng/ml), indicating reduced iron stores. The rate increased to 18% (37 out of 206 individuals) among regular blood donors (Groups III, IV, and V). Iron deficiency (depletion) prevalence among regular blood donors in Groups III, IV, and V was 5.9% (12 out of 206) and 50.4% (100 out of 206). Donors who had donated blood most frequently had the lowest levels of haematological markers MCH, MCHC, and TIBC. Provide the p-values representing the differences between the means of MCV, MCH, iron, TIBC, and ferritin levels when comparing donor groups with the control group (Group I) based on the frequency of donations. Indicate statistically significant differences where the p-value is less than 0.0125. This significance level is adjusted based on the Bonferroni method, considering multiple independent tests. The result shows that the Iron parameter for the comparison between Group I and Group III and Group I and

Group IV suggests a statistically significant difference in iron levels between these donor groups. **Conclusion:** The findings of this study show that a higher times of donations leads to a higher occurrence of depleted iron stores and subsequent erythropoiesis with iron deficiency by one donor from every three healthy donors. The iron and ferritin concentrations were within the normal range in group one (Control group) and reduced in the other four groups (G-2 to G-5). However, the level of haemoglobin remained within an acceptable range for blood donation. This outcome suggests that it may be necessary to reassess the criteria for accepting blood donors. The average serum ferritin levels were examined in all five groups (G-1 to G-5), both for males and females, and significant variations were seen among the groups under study. This study found that 35% of the individuals who regularly donate blood have iron-deficient anaemia (sideropenia). This suggests that it would be beneficial to test for serum ferritin at an earlier stage, ideally after three donations.

### Keywords

Iron Deficiency Anaemia, Regular Blood Donors, Anaemia, Volunteer Blood Donor, Blood Donation

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## 1. Introduction

Iron deficiency is the most distinct and singular deficit of a nutrient globally, and it is also the primary cause of anaemia [1]. In cases of iron deficiency anaemia, the concentration of haemoglobin in the blood is decreased to levels below 120 g/L for females and below 130 g/L for males [2].

Iron deficiency without anaemia is a prevalent condition. It can be severe even if the levels of haemoglobin and the results of a complete blood count (CBC) are within the normal range. According to predictions, individuals who have been deferred as blood donors due to their low haemoglobin levels provide 30% less blood over the next four to five-year period, even after their haemoglobin levels return to normal, compared to those who have not been deferred [3].

Factors other than blood-related issues contribute to iron deficiency, and these non-haematological causes can result in unpleasant symptoms, such as weariness, low physical stamina, difficulties in maintaining body temperature, poor cognitive function and more. Analyzing the aetiology of iron insufficiency is crucial, as it is not a standalone ailment but rather a manifestation of an underlying issue [4]. Ferroportin facilitates the transportation of iron from cells into the bloodstream, where it binds to transferrin and is transported to macrophages. Ferritin also increases storage capacity [5].

Serum ferritin is the most versatile diagnostic test for determining iron deficiency. Central hypoxia can lead to symptoms, such as dyspnea, dizziness, and cognitive impairment, as well as a pale complexion. However, after anaemia is treated, the paleness may improve. This study confirmed that suppressed iron deficiency anaemia (IDA) severely impacts the quality of life and determined the

cause of donation whole blood process failed to complete after three to five minutes in those within accepted hemoglobin donation range due to fatigue onset (QoL) [6] [7].

A 450 mL blood donation, along with an extra 50 mL for laboratory tests, from a donor with a haemoglobin (Hb) level of 12.5 g/dl will lead to a loss of 200 mg of iron [8] [9].

Repeated blood donations provide a risk of iron deficiency (ID) for regular blood donors, characterised by low serum ferritin levels [10]. Hence, it is imperative to safeguard blood donors from these hazards. Recent evidence has demonstrated the validity of employing iron status as a basis for donor deferral policy, which effectively safeguards donors from iron deficiency [11] [12].

Haemoglobin, serum ferritin, transferrin saturation, and erythrocyte protoporphyrin are all methods used to quantify body iron reserves.

Iron deficiency is frequently the cause of anaemia. Its primary causes are primarily menstruation-related bleeding and gastrointestinal bleeding. Iron supplements can be administered either orally or intravenously to those for whom oral iron is ineffective or contraindicated [13] [14].

The purpose of the minimal Hb threshold is to avoid collecting blood from donors who have anaemia, but it does not prevent the collection of blood from donors who have iron deficiency. Regular blood donors are the primary providers of life-saving blood for critical medical conditions. Their primary objectives are to save lives, and blood donations are used in various medical settings, such as birthing rooms, pediatric departments, adult and neonatal intensive care units, emergency rooms, critical care units, operating rooms and surgical units [15].

In this research, the study sample comprises 300 male and female donors who visited the blood donation center and were attached to a 300-bed facility. Starting on October 1, 2023, and lasting until February 2024, data will be collected and classified weekly for five months. This will be done after obtaining ethical approval from the institutional ethics council and the Jazan University ethical research scientific ethical committee. The categorical data was analyzed using descriptive statistics, frequencies, and percentages to demonstrate the variations in percentages across five groups based on the features of various laboratory test factors. A chi-squared test was conducted to compare categorical data. A unidirectional analysis of variance (ANOVA) was used to assess the impact of various donors on the depletion of IBS and levels of Iron over the past four years. When a significant overall effect was seen, a post hoc test was performed to identify the specific donor groups that contributed to this effect. Ordinal linear regression analysis was used to determine the disparities among various categories of donors. Ordinal regression is a statistical method used to find out the dependent variable iron store reserves, which has three levels: normal, decreased, depleted, and iron serum sideropenia. This prediction is based on a collection of independent factors. The dependent variable refers to the variable that is being measured or observed in relation to the order of response categories. The independent variable, on the

other hand, can either be categorical or continuous. The analyses were performed using SPSS version 22.0 (IBM Corp., New York, NY, USA). A significance level of  $P < 0.05$  was employed to determine statistical significance.

## 2. Materials and Methods

A scientific prospective study focused on the blood bank and laboratory services at Beish G-H and Jazan Nursing and Health Science Hospital University provided to these distinguished ABO Rh donor types, which included medical support after obtaining written and verbal consent from the volunteer blood donors at Beish G. H blood bank donation center. The study also examined the frequency of blood donations and the responses to a brief deferral questionnaire given to regular blood donors who visited the blood donation center. The work received approval from the Standing Committee for Scientific Research—Jazan University (HAPO-10-Z-001). Participants were selected from October 2023 to February 2024. Blood samples were collected from 300 volunteer blood donors; each donor was given  $450 \pm 50$  mL of whole blood for further testing. As soon as the donation was made, samples were collected into 2.5 mL EDTA (Ethylene diamine tetra acetic acid) tubes sample (purple-top) for measurement of RBCs Indices and into three mL plain tubes (yellow-top) collected for this study after obtaining approval from regular volunteer donors. The serum was separated and transferred into plain vials and stored at  $-20^{\circ}\text{C}$  to estimate iron, total iron binding capacity, and donor ferritin accumulation. Haemoglobin is also measured spectrophotometrically at the Sysmex XN-550-L-Series automated cell counter on blood samples (2.5 mL) containing anti-coagulant EDTA to accept or reject those donors based on the reality update last read of their haemoglobin volume with red blood cells indices. Separated serum was used to measure serum iron concentrations. 500  $\mu\text{l}$  of Serum was dispensed on a plastic ROCH cuvette; serum was run, and iron was estimated using a BECKMAN COULTER DXC-700-AU Clinical Biochemistry Analyzer (CA, U.S.A) as per the manufacturer's instructions.

### 2.1. Haematological and Biochemical Parameter Analysis

- 1) CBC Machine Sysmex (Complete Counter Blood Count) Model Number Sysmex XN-550-L-Series (Coulter, Japan);
- 2) FRESINIUS KABI Compo-lab TM 61346 model (Hamburg, Germany);
- 3) BECKMAN COULTER DXC-700-AU (CA, U.S.A);
- 4) Thermo Electron LED GmbH D-37520 Osterode (Made in Germany). Donor's Collection;
- 5) BIORAD IH-500 Tested Donors ABO Rh (Made in USA) confirm ABO Rh donors;
- 6) BECKMAN COULTER ACCESS 2 (CA, U.S.A).

### 2.2. Study Design

A scientific prospective study was conducted at Beish General Hospital and Jazan

University Hospital, a tertiary care hospital with 150 beds, to investigate voluntary whole-blood donors. The study focused on the blood bank and laboratory services provided to these distinguished ABO Rh donor types, which included medical support after obtaining written and verbal consent from the Saudi volunteers. The study also examined the frequency of blood donations and the responses to a brief deferral given to regular blood donors who visited the blood donation center. The work received approval from the Standing Committee for Scientific Research—Jazan University (HAPO-10-Z-001). Participants were selected from October 2023 to February 2024.

### **Donor Selection Criteria**

The donors were queried with particular inquiries on blood donation, following the guidelines outlined in the technical manual of the Directorate General of Health directions Saudi Health Ministry. The selected donors were queried about their age at the first donation, last donation date, number of donations made in the past four years, time intervals between donations, dietary history, medication usage, educational background, and dosage of iron supplementation for those who are knowledgeable about its use. Additionally, information was collected regarding the use of hormone therapy, such as testosterone and growth factors among bodybuilders (if applicable).

#### **1) Inclusion Criteria**

The study encompassed all eligible voluntary donors (both male and female) who had made donations over the prior four years. The most recent contribution had to be within the previous year, with a minimum interval of 90 days (or 3 months), and the donors had to maintain a consistent pattern of donating at least once each year.

The contributors included in the study were subsequently categorized into five distinct groups based on their number of donations, Group I consists of donors who made their first donation, Group II consists of donors who made two donations, Group III consists of donors who made three to four donations, Group IV consists of donors who made five to nine donations, and Group V consists of donors who made 10 to 16 donations in the last four years.

#### **2) Exclusion Criteria**

- a) All voluntary donors who did not fulfil the criteria of healthy and safe donors.
- b) Trait HBAS (Carrier—S Hemoglobin).
- c) All Donors who consumed iron supplemental, growth hormone therapy on the gem program.

### **2.3. Study Methodology**

Blood samples were collected from 300 volunteer blood donors; each donor was given  $450 \pm 50$  mL of whole blood for further testing. As soon as the donation was made, samples were collected into 2.5 mL EDTA (Ethylene diamine tetra acetic acid) tubes sample (purple-top) for measurement of RBCs Indices and into three mL plain tubes (yellow-top) collected for this study after obtaining approval from

regular volunteer donors. The serum was separated and transferred into plain vials and stored at  $-20^{\circ}\text{C}$  to estimate iron, total iron binding capacity, and donor ferritin accumulation.

### **2.3.1. Haemoglobin Estimation**

Haemoglobin levels were estimated for 300 volunteer blood donors. After the donor's sterilizing finger breaking with the lancet, one blood drop was taken on a specific plastic clear slide design at the donation unit using the FRESINIUS KABI Compo-lab TM 61346 model (Hamburg, Germany). Haemoglobin is also measured spectrophotometrically at the Sysmex XN-550-L-Series automated cell counter on blood samples (2.5 mL) containing anti-coagulant EDTA to accept or reject those donors based on the reality update last read of their haemoglobin volume. Reference haemoglobin to accept blood donors for males (13 g/dl - 15.5 g/dl) and for females (12.5 g/dl to 14.5 g/dl).

### **2.3.2. Red Cell Indices Calculation**

Blood samples from the study subjects were collected for haematological tests: MCV (Mean Corpuscular Volume), MCH (Mean Corpuscular Haemoglobin), and MCHC (Mean Corpuscular Haemoglobin Concentration). Red cell indices were measured by using Sysmex XN-550-L-Series (Coulter, Japan). A normal range of:

MCV is 80 - 100 fl.

MCH is 27 to 31 pg.

MCHC for an adult is 32 - 36 g/dl.

### **2.3.3. Assessment of Iron**

Three hundred regular blood donors were prospectively included in this study. All selected donors were healthy according to their clinical histories and physical examinations and fulfilled the suitability criteria for haemoglobin donation to accept blood donors for males (13 g/dl to 15.5 g/dl) and females (12.5 g/dl to 14.5 g/dl).

Separated serum was used to measure serum iron concentrations. 500  $\mu\text{l}$  of serum was dispensed on a plastic ROCH cuvette; serum was run, and iron was estimated using a BECKMAN COULTER DXC-700-AU Clinical Biochemistry Analyzer (CA, U.S.A) as per the manufacturer's instructions.

### **2.3.4. Assessment of Ferritin**

All three hundred regular blood donors were prospectively included in this study; a separate serum was used to measure serum iron store concentrations. 1000  $\mu\text{l}$  of serum was dispensed on a plastic ROCH cuvette; serum was run, and iron store (Ferritin) was estimated using a BECKMAN COULTER ACCESS 2 (CA, U.S.A) as per the manufacturer's instructions.

### **2.3.5. Calculation of Total Iron-Binding Capacity (TIBC)**

Total iron-binding capacity (TIBC) is an essential test used for the diagnosis of

iron deficiency anaemias and other disorders of iron metabolism, TIBC value reporting by BECKMAN COULTER DXC-AU 700 software as iron profile result. Iron binding capacity is the capacity of transferrin to bind with iron. The principle of measuring the blood donor TIBC is by first saturating the serum of donor transferrin with excess  $\text{Fe}^{3+}$ .

Total iron binding capacity (TIBC) was calculated by the formula:

$$\text{TIBC } (\mu\text{g/dl}) = \text{Transferrin } (\text{mg/dl}) \times 1.25^{21}$$

(Another reference = Transferrin m g/dl  $\times$  0.025) to convert m g/dl to  $\mu\text{mol/l}$ , multiply by 88.4. The reference range value for TIBC was 255 - 450 m g/dl and average (65 - 176), transferrin saturation (TSAT-Transferrin saturation) was calculated by the formula:

$$\text{TSAT} = (\text{Serum iron/TIBC}) \times 100\%$$

(To convert m g/dl to  $\mu\text{mol/l}$ , multiply by 88.4).

Donors were divided into five groups: Group I, donors who donated for the first time; Group II, donors who donated twice; Group III, donors who donated 2 - 4 times; Group IV, donors who donated 5 - 9 times; and Group V, donors who donated 10 - 16 times in the last four years.

Iron stores (Ferittin) were considered depleted at serum ferritin values  $< 12$  ng/ml, reduced at values between 15 - 24 ng/ml, and normal or replete at values from 24 - 337 ng/ml, and increased at values beyond 300  $\mu\text{g/dl}$ . Iron deficiency anaemia was considered present when serum ferritin was  $< 12$  ng/ml.

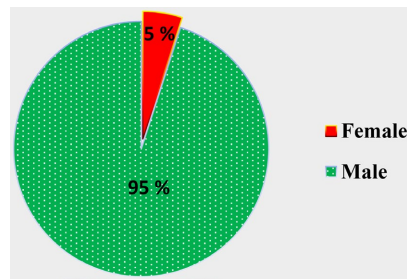
All three hundred regular blood donors were prospectively included in this study; a separate serum was used to measure serum iron store concentrations. 1000  $\mu\text{l}$  of serum was dispensed on a plastic ROCH cuvette; serum was run, and iron store (Ferittin) was estimated using a BECKMAN COULTER ACCESS 2 (CA, U.S.A) as per the manufacturer's instructions. The analyses were performed using SPSS version 22.0 (IBM Corp., New York, NY, USA). A significance level of  $P < 0.05$  was employed to determine statistical significance.

### 3. Results

The 300 donors included in this study included 285 males (95%) and 15 females (5%) (Table 1 and Figure 1). The participant women were 22 to 48 years old. The age range of the men cases was between 17 and 51, with an overall mean age for both groups of  $33 \pm 8$ . The majority of the donors in the study sample are male (95%) Table 1 and Figure 1.

**Table 1.** Displays the distribution of males and females in the study sample.

Gender	Frequency	Percent
Female	15	5.0
Male	285	95.0
Total	300	100.0



**Figure 1.** Gender distribution of voluntary blood donors, N = 300.

Thirty-six donors (12%) were first-time donors with no previous history of blood donations, and 264 donors (88%) had donated at least once in the previous three years. The population under study was divided into five groups according to the number of previous donations in the last four years (**Table 2**). The blood donors in Group II donated on an average of one to two times a year and were considered regular donors. There are 58 donors in this group, representing 19.3% of the total donors. The result shows that Group III consists of donors who have made 3 to 4 donations. There are 79 donors in this group, making up 26.3% of the total donors. Donors in group IV have made 5 to 9 donations. There are 60 donors in this group, accounting for 20.0% of the total donors. Group V includes donors who have made 10 to 16 donations. There are 67 donors in this group, representing 22.3% of the total donors. Donors who have not made any donations (group I) at (**Table 3**) have slightly higher MCV and MCH values compared to other groups, indicating larger red blood cells with more haemoglobin content. Iron and ferritin concentrations are also relatively higher in this group. Group II: Donors show light variations in blood parameters from Group I, with a slight decrease in MCV and MCH values and fluctuations in iron and ferritin concentrations.

**Table 2.** Distribution of donors according to their age.

Groups	Number of Donation	Number of Donors	Age (years) Mean (Range)	Standard Deviation	%
Group I	0	36	23 (17 - 41)	6.4	12.0
Group II	1 - 2	58	31 (19 - 50)	7.9	19.3
Group III	3 - 4	79	33 (21 - 51)	7.2	26.3
Group IV	5 - 9	60	35 (22 - 50)	6.6	20.0
Group V	10 - 16	67	37 (21 - 51)	6.4	22.3
Total		300	33 (17 - 51)		100.0

**Table 3.** Shows the Mean values of iron and ferritin concentrations, MCH, MCHC, MCV and TIBC in donors grouped according to frequency of donation.

Groups	Nr. of Donation	MCV (fL)	MCH (pg/cell)	MCHC (g/dl)	Iron ( $\mu\text{mol/L}$ )	TIBC ( $\mu\text{mol/L}$ )	Ferritin (ng/ml)
Group I	0	89.3 $\pm$ 6.2	26.9 $\pm$ 1.9	29.9 $\pm$ 2.8	32.3 $\pm$ 4.5	35.3 $\pm$ 7.3	89 $\pm$ 60.7

## Continued

Group II	1 - 2	87.1 ± 9.8	26.7 ± 2.5	29.2 ± 3.1	25.9 ± 14.8	42.9 ± 12.2	82 ± 76.8
Group III	3 - 4	89.2 ± 12.7	26.6 ± 2.7	28.9 ± 5.6	26.2 ± 19.5	45.7 ± 13.8	57.9 ± 77.4
Group IV	5 - 9	89.2 ± 10.5	26.2 ± 2.4	27.4 ± 2.8	16.2 ± 10.1	50.4 ± 15.5	19 ± 17.6
Group V	10 - 16	89.1 ± 10.8	26 ± 2.5	27.5 ± 2.8	8.2 ± 4.5	58.4 ± 13.8	8.9 ± 5.9

The values are given as \*Mean ± Standard deviation.

The blood parameters for Group III show similar trends to Group II, with some fluctuations in MCV, MCH, iron, and ferritin concentrations.

Meanwhile, the blood parameters of Group IV exhibit a slight decrease in MCV, MCH, iron, and ferritin concentrations compared to previous groups.

The results in (Table 3) and (Figures 2-7) indicate that for Group V, the blood parameters show further decreases in MCV, MCH, iron, and ferritin concentrations, suggesting potential effects of frequent blood donation on these parameters, especially for ferritin.

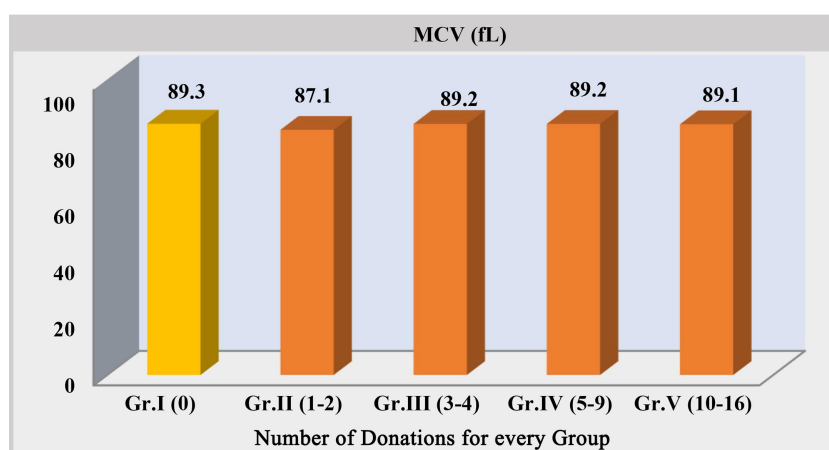


Figure 2. MCV concentrations of blood donors according to the frequency of donation.

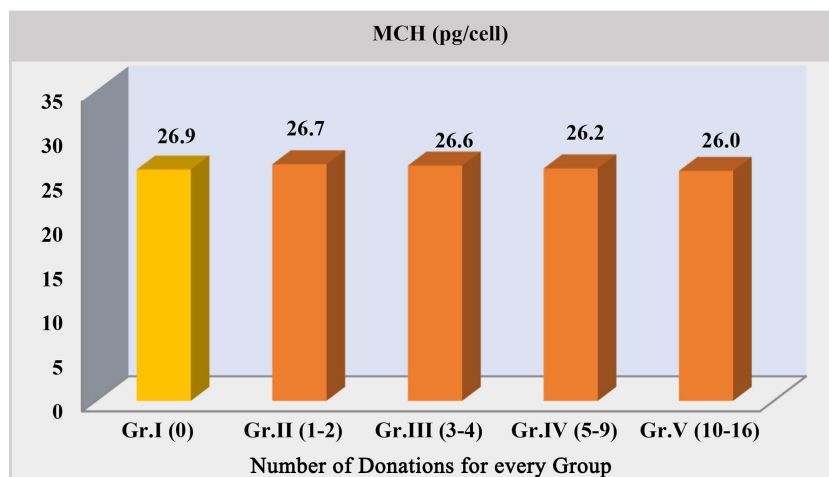
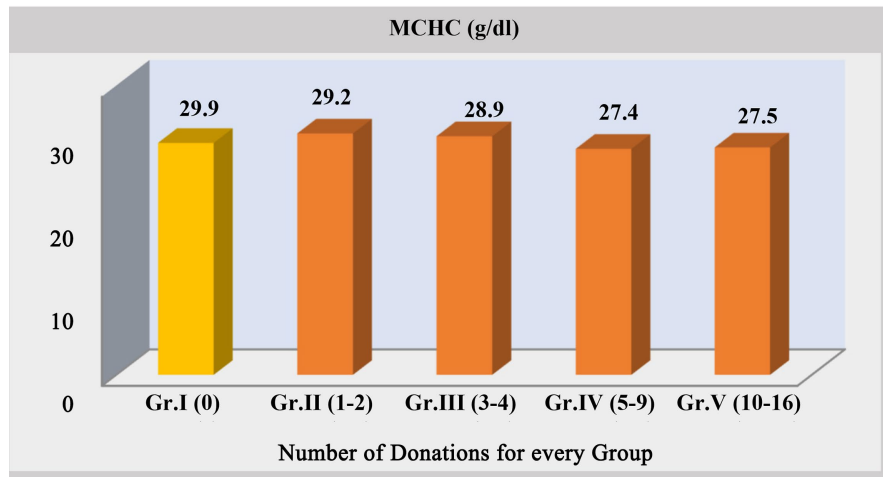
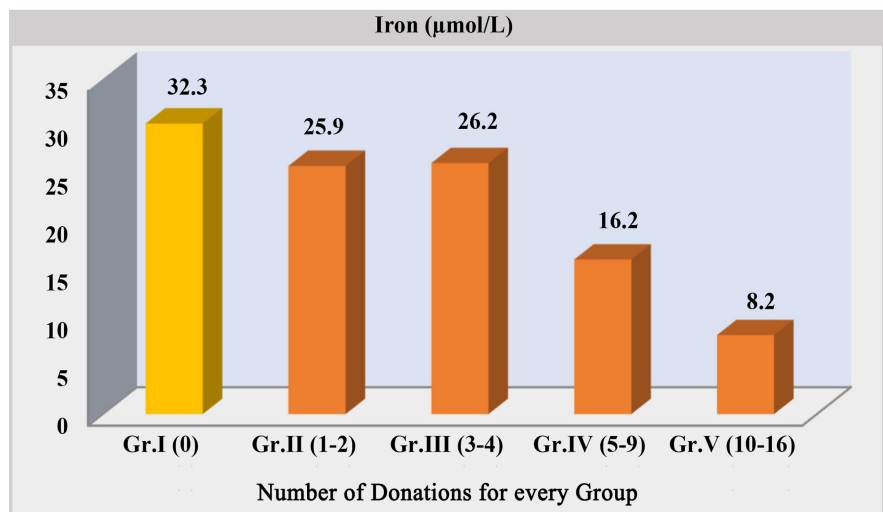


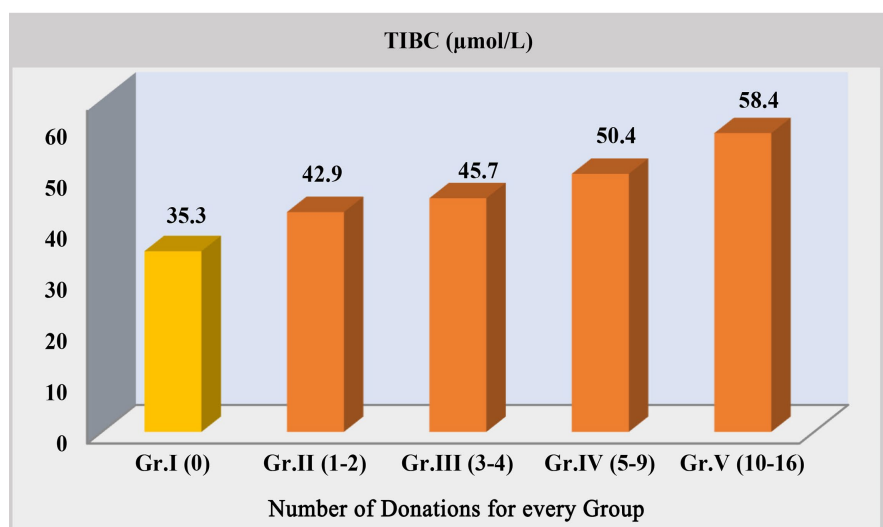
Figure 3. MCH concentrations of blood donors according to frequency of donation.



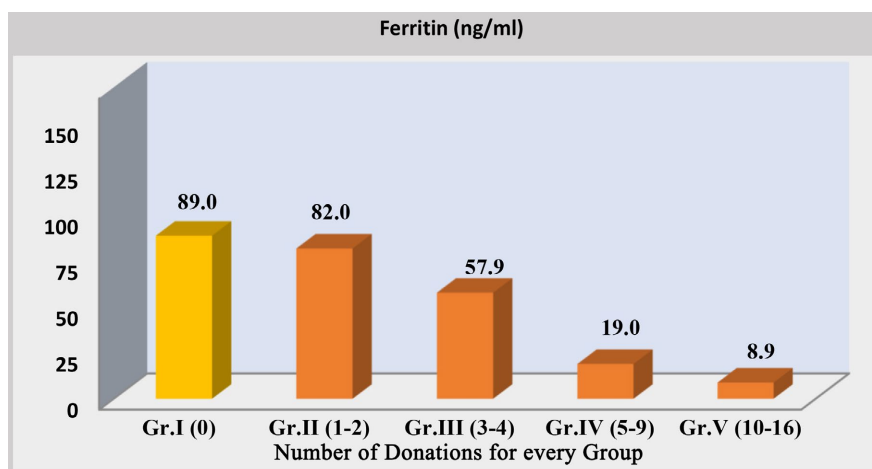
**Figure 4.** MCHC concentrations of blood donors according to the frequency of donation.



**Figure 5.** Iron concentrations of blood donors according to frequency of donation.



**Figure 6.** TIBC concentrations of blood donors according to frequency of donation.



**Figure 7.** Ferritin concentrations of blood donors according to frequency of donation.

The results presented in (Table 4) provide the p-values representing the differences between the means of MCV, MCH, iron, TIBC, and ferritin levels when comparing donor groups with the control group (Group I) based on the frequency of donations. Here's the interpretation. Cells marked with "\*" indicate statistically significant differences where the p-value is less than 0.0125. This significance level is adjusted based on the Bonferroni method, considering multiple independent tests.

**Table 4.** Statistical analyses with P-values of the differences between means of MCV, MCH, iron, TIBC, and ferritin when comparing donor groups with control (Group I) groups according to the frequency of donations.

Difference between the Control Group I and the Other 4 Groups	P-Values for Each Factor					
	MCV	MCH	MCHC	Iron	TIBC	Ferritin
Gr. I: Gr. II (0: 1 - 2)	0.188	0.197	0.070	0.035	0.003*	0.650
Gr. I: Gr. III (0: 3 - 4)	0.965	0.315	0.423	0.078	0.000*	0.035
Gr. I: Gr. IV (0: 5 - 9)	0.963	0.793	0.224	0.000*	0.000*	0.000*
Gr. I: Gr. V (0: 10 - 16)	0.905	0.743	0.345	0.000*	0.000*	0.000*

\*Represents statistically adjusted significant value ( $P < 0.0125$ ) based on the Bonferroni method in conducting more than two independent tests. The significance level of 0.05 was divided by 4 as we compared control group I four times with other groups II and group V.

In Group I (0 donations), most donors have normal iron stores (91.7%), followed by a small percentage with reduced iron stores 5.6% (2/36) (Table 4).

As the number of donations increases (Groups II to V), the percentage of donors with normal iron stores decreases gradually, while the percentage of donors with reduced, depleted, and severely deficient iron stores increases.

Notably, in Group V (10 - 16 donations), only a small percentage of donors (1.5%) have normal iron stores, while the majority exhibit signs of iron deficiency, with a significant portion (77.6%) categorised as severely deficient.

Overall, the table illustrates a correlation between the frequency of blood donations and the status of iron stores, with frequent donors more likely to exhibit iron deficiency.

To analyze the relationship between the dependent variable, which indicates iron deficiency through using “Ferritin concentration” with four levels, Normal > 24 ng/ml, Reduced iron (15 - 24 ng/ml), Depleted iron (<12 ng/ml) and sideropenia (<10.70 umol/l) and, the cumulative odds ordinal logistic regression with proportional odds was used, which uses cumulative categories.

**Table 5** shows that the odds of donors increasing one unit of their iron level are 0.82 (95% CI, -0.79 to -0.86) times less likely to be in a higher category of “Ferritin concentration”. This means that there is an 18% chance that the donors will be in the normal category. Wald  $\chi^2(1) = 80.61$ ,  $P < 0.001$ .

**Table 5.** The odds ratio values (Exp (b)) and their 95% confidence intervals for assessing significant predictors of age, Iron level, TIBC, MCV, MCH, and groups of donations for possibly affecting the concentration level of iron for the donors.

Predictor	Estimate (b)	Std. Error	Wald	df	Sig	Exp (b)	95% CI	
Age (Years)	-0.03	0.02	2.39	1	0.122	0.97	0.93	1.01
IRON level	-0.20	0.02	80.61	1	<b>0.000*</b>	0.82	0.79	0.86
TIBC	0.03	0.01	6.07	1	<b>0.014*</b>	1.03	1.01	1.05
MCV	-0.02	0.01	1.19	1	0.276	0.98	0.96	1.01
MCH	0.06	0.06	0.77	1	0.379	1.06	0.93	1.19
<b>Gr. II (1 - 2)</b>	0.34	0.84	0.16	1	0.687	1.40	0.27	7.30
<b>Gr. III (3 - 4)</b>	1.47	0.82	3.20	1	0.074	4.34	0.87	21.67
<b>Gr. IV (5 - 9)</b>	2.68	0.83	10.42	1	<b>0.001*</b>	14.56	2.86	74.05
<b>Gr. V (10 - 16)</b>	2.75	0.91	9.23	1	<b>0.002*</b>	15.69	2.66	92.62

Whereas for those individuals whose “TIBC” level increases, the results suggest that they are 1.03 (95% CI, 1.01 to 1.05) times more likely to be in the higher category of the ordinal outcome, *i.e.*, sideropenia, compared to those with decreasing TIBC, a statistically significant effect, Wald  $\chi^2(1) = 6.07$ ,  $P = 0.0014$ .

#### 4. Discussion

The current study examined the occurrence of iron deficiency anaemia in a group of 300 individuals who regularly donate blood based on age mean in study group. Of these, 285 (95%) were male and 15 (5%) were female. The average age for both male and female blood donors is 33 +/- 8 years, with male donors ranging from 17 to 51 years and female donors ranging from 22 to 48 years. The finding is not statistically significant, suggesting that gender and age did not have a significant

impact on the development of iron deficiency anaemia.

The study population was categorized into five groups based on the frequency of previous donations within the past four years. The blood donors in Group 1, also known as the Control group, had not previously donated in the past four years and provided blood for the first time. On the other hand, the blood donors in the other groups (Group 2 to Group 5) donated blood on a regular basis, with an average frequency ranging from 2 to 16 times over the past four years.

The average serum ferritin levels were examined in all five groups (G-1 to G-5), both for males and females, and significant variations were seen among the groups under study.

Long-term blood donors frequently show chronic iron insufficiency, as observed in several studies [16]-[18]. Iron deficiency refers to a condition when the amount of iron in the body is below the usual level. The current screening test used to determine the eligibility of a blood donor for donation is the Hemoglobin (Hb) test. The purpose of the minimal Hb threshold is to avoid collecting blood from donors who have anaemia, but it does not prevent the collection of blood from donors who have iron deficiency.

This study found that 35% of the individuals who regularly donate blood have iron-deficient anaemia (sideropenia).

The iron and ferritin concentrations were within the normal range in group one (Control group) and reduced in the other four groups (G-2 to G-5).

Regular blood donations are known to reduce iron stores (Sideropenic Aneamia). Published studies have used various indicators to identify iron deficiency and depletion in blood donors [19]-[21].

The study findings indicate a significant drop in ferritin concentrations as the frequency of the number of donations increased. The findings are similar to other findings with the conclusions documented by other researchers [22]-[25].

In this investigation, approximately 15.5% of individuals in Group II who had donated blood once exhibited ferritin levels ranging from 15 - 30 ng/mL, indicating a decrease in iron reserves (**Table 6**). The rate rose to 18% (37 out of 206) among regular blood donors (Groups III, IV, and V). The prevalence of iron deficiency (depletion) among regular blood donors (Groups III, IV, and V) was 5.9% (12 out of 206), and 50.4% (100 out of 206) of the regular donors (groups III, IV, and V) exhibited erythropoiesis with iron deficiency (sideropenia). This study additionally demonstrates that a rise in the number of donations leads to a higher occurrence of diminished iron reserves and, as a result, erythropoiesis with iron deficiency.

The total iron-binding capacity (TIBC) test measures the quantity of iron that can be attached to unsaturated transferrin. Nevertheless, it has been stated that the haematological parameters indicate that (TIBC) is not a reliable indicator of early iron shortage since its values do not change until iron stores are completely depleted [26]. This study found statistically significant changes in (TIBC) as the number of donations increased.

**Table 6.** The relationship between iron stores' status and the number of donations.

Donors Groups	Iron Stores for the Donor's Participants Value Given as n (%)				Number of Donors in Each Group n (%)
	Normal > 24 ng/ml	Reduced 24 - 12 ng/ml	Depleted < 12 ng/ml	Erythropoiesis with iron deficiency (sideropenia) < 10.7 umol/L	
Group I (0)	33 (91.7%)	2 (5.6%)	1 (2.8%)	0 (0%)	36 (100%)
Group II (1 - 2)	43 (74.1%)	9 (15.5%)	1 (1.7%)	5 (8.6%)	58 (100%)
Group III (3 - 4)	43 (54.4%)	17 (21.5%)	3 (3.8%)	16 (20.3%)	79 (100%)
Group IV (5 - 9)	13 (21.7%)	12 (20%)	3 (5%)	32 (53.3%)	60 (100%)
Group V (10 - 16)	1 (1.5%)	8 (11.9%)	6 (9%)	52 (77.6%)	67 (100%)
Total	133 (44.3%)	48 (16%)	14 (4.7%)	105 (35%)	300 (100%)

The average \* ferritin levels of regular blood donors showed a considerable drop after just five donations. This suggests that it would be beneficial to test for serum ferritin at an earlier stage, ideally after three donations.

The level of iron store (ferritin) depletion in the various groups increased proportionally with the increase in the number of donations. Specifically, group III saw a 3.8% increase, group IV experienced a 5% increase, and group V experienced a 9% increase. These findings are in accordance with previous studies [19]-[29].

In conclusion, this study demonstrates a significant occurrence of diminished iron stores, particularly among individuals who donate blood often despite their eligibility to do so. Implementing measures that more precisely indicate iron status, such as serum iron concentration and ferritin level, would enhance the safety, sufficient and reproductability of the blood donation procedure for all donors.

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## Conflicts of Interest

The authors have no potential conflicts of interest.

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