

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

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Abstract

Chronic iron insufficiency is a common observation in long-term blood donors, and it can lead to anaemia. Iron-status markers are not often included in the majority of blood screening procedures used by blood banks, which could lead to subclinical iron deficiency. This study aimed to assess the impact of repeated blood donation on the body's iron levels and provide recommendations to blood donors on how to avoid the body's iron reserves being depleted. Techniques: The association between the iron levels in these groups and their bodies was investigated after regular blood donors were divided into discrete groups based on the quantity of blood they donated. Serum ferritin, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), total iron-binding capacity (TIBC), and serum iron were among the characteristics used to determine iron deficiency and iron depletion in blood donors. Findings: A total of 300 consenting and frequent blood donors were involved in the study. Among individuals giving blood for the first time, there were no occurrences of iron inadequacy (Group I). However, 15.5% of those who had given once before (Group II) had ferritin levels of 15 - 30 µg/dl (ng/ml), indicating lower iron reserves. The percentage among regular blood donors (Groups III, IV, and V) rose to 18% (37 out of 206 individuals). In Groups III, IV, and V, the prevalence of iron deficiency (depletion) was 5.9% (12 out of 206) and 50.4% (100 out of 206) among frequent blood donors. The blood donors with the lowest levels of haematological indicators MCH, MCHC, and TIBC were the most often donated blood donors. When comparing the donor groups with the control group (Group I) based on the frequency of donations, provide the p-values indicating the differences between the means of MCV, MCH, iron, TIBC, and ferritin levels. Declare differences that are statistically significant when the p-value is less than 0.0125 (Abdullah, 2011). The Bonferroni procedure is used to alter this significant level while taking into account several independent tests. The outcome indicates that there may have been a statistically significant variation in the iron levels between these donor groups based on the comparison of the Iron Parameter between Group I and Group III and Group I and Group IV. In summary: According to the study's findings, one out of every three healthy donors will experience erythropoiesis with iron insufficiency as a result of depleted iron storage, which is more common in higher donation frequencies. Group I (Control group) had iron and ferritin concentrations within the normal range, however the other four groups (G-2 to G-5) had lower concentrations. The hemoglobin level, however, stayed within a range that is suitable for blood donation. This result implies that reevaluating the requirements for acceptance may be essential. Significant differences were seen between the five groups (G-1 to G-5) when the average serum ferritin levels were analyzed for both males and females. According to this study, 35% of those who routinely give blood suffer with sideropenia, or iron-deficient anemia. This implies that serum ferritin testing must be done sooner rather than later, ideally following three donors (Norashikin et al., 2006).

نقص الحديد المزمن هو ملاحظة شائعة لدى المتبرعين بالدم على المدى الطويل، ويمكن أن يؤدي إلى فقر الدم. لا يتم تضمين علامات حالة الحديد غالبًا في غالبية إجراءات فحص الدم التي تستخدمها بنوك الدم، مما قد يؤدي إلى نقص الحديد دون السرييري. هدفت هذه الدراسة إلى تقييم تأثير التبرع المتكرر بالدم على مستويات الحديد في الجسم وتقديم توصيات للمتبرعين بالدم حول كيفية تجنب استنفاد احتياطي الحديد في الجسم. التقنيات: تم التحقيق في العلاقة بين مستويات الحديد في هذه المجموعات وأجسامهم بعد تقسيم المتبرعين بالدم المنتظمين إلى مجموعات منفصلة بناءً على كمية الدم التي تبرعوا بها. كان فيريتين المصل، ومتوسط حجم الكريات (MCV)، ومتوسط الهيموجلوبين الكروي (MCH)، ومتوسط تركيز الهيموجلوبين الكروي (MCHC)، والقدرة الكلية على ربط الحديد (TIBC)، وحديد المصل من بين الخصائص المستخدمة لتحديد نقص الحديد واستنفاد الحديد لدى المتبرعين بالدم. النتائج: شارك في الدراسة ما مجموعه 300 متبرع بالدم موافق ومتكرر. بين الأفراد الذين تبرعوا بالدم لأول مرة، لم تحدث حالات نقص الحديد (المجموعة الأولى). ومع ذلك، كان لدى 15.5% من أولئك الذين تبرعوا مرة واحدة من قبل (المجموعة الثانية) مستويات فيريتين تتراوح من 15 إلى 30 ميكروغرام / ديسيلتر (نانوغرام / مل)، مما يشير إلى انخفاض احتياطي الحديد. ارتفعت النسبة بين المتبرعين بالدم المنتظمين (المجموعات الثالثة والرابعة والخامسة) إلى 18% (37 من 206 فردًا). في المجموعات الثالثة والرابعة والخامسة، كان معدل انتشار نقص الحديد (الاستنزاف) 5.9% (12 من 206) و 50.4% (100 من 206) بين المتبرعين بالدم المنتظمين. كان المتبرعون بالدم الذين لديهم أدنى مستويات المؤشرات الدموية MCH و MCHC و TIBC هم المتبرعين بالدم الأكثر تكرارًا. عند مقارنة مجموعات المتبرعين بمجموعة التحكم (المجموعة الأولى) بناءً على تكرار التبرعات، قم بتوفير القيم الاحتمالية التي تشير إلى الاختلافات بين متوسطات مستويات MCV و MCH والحديد و TIBC والفيريتين. أعلن الاختلافات ذات الدلالة الإحصائية عندما تكون القيمة الاحتمالية أقل من 0.0125 (عبد الله، 2011). يتم استخدام إجراء بونفيروني لتغيير هذا المستوى الهام مع مراعاة العديد من الاختبارات المستقلة. تشير النتيجة إلى أنه ربما كان هناك اختلاف ذو دلالة إحصائية في مستويات الحديد بين مجموعات المتبرعين هذه بناءً على مقارنة معامل الحديد بين المجموعة الأولى والمجموعة الثالثة والمجموعة الأولى والمجموعة الرابعة. باختصار: وفقًا لنتائج الدراسة، سيعاني واحد من كل ثلاثة متبرعين أصحاب من تكون كريات الدم الحمراء مع نقص الحديد نتيجة لاستنفاد تخزين الحديد، وهو أكثر شيوعًا في تواتر التبرع الأعلى. كانت تركيزات الحديد والفيريتين في المجموعة I (مجموعة الضبط) ضمن النطاق الطبيعي، إلا أن المجموعات الأربع الأخرى (G-2) إلى (G-5) كانت تركيزاتها أقل. ومع ذلك، ظل مستوى الهيموجلوبين ضمن النطاق المناسب للتبرع بالدم. وتشير هذه النتيجة إلى أن إعادة تقييم متطلبات القبول قد تكون ضرورية. وقد لوحظت فروق كبيرة بين المجموعات الخمس (G-1) إلى (G-5) عندما تم تحليل مستويات الفيريتين المتوسطة في المصل لكل من الذكور والإناث. ووفقًا لهذه الدراسة، فإن 35% من أولئك الذين يتبرعون بالدم بشكل روتيني يعانون من نقص الحديد، أو فقر الدم الناجم عن نقص الحديد. وهذا يعني أن اختبار الفيريتين في المصل يجب أن يتم في أقرب وقت ممكن، ومن الأفضل بعد ثلاثة متبرعين. (Norashikin et al., 2006).

Keywords

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

كلمات المفتاحية

فقر الدم الناجم عن نقص الحديد، المتبرعون بالدم بانتظام، فقر الدم، المتبرعون المتطوعون بالدم، التبرع بالدم.

Introduction

The most notable and unique dietary deficiency in the world is iron deficiency, which is also the main cause of anemia (Lopez et al., 2016). The blood's hemoglobin concentration falls to less than 120 g/L for females and less than 130 g/L for males when iron deficiency anemia is present (Mei & Grummer-Strawn, 2019).

The state of iron insufficiency without anemia is common. Even if the complete blood count (CBC) and hemoglobin levels are within normal limits, the condition may nevertheless be severe. Even once their hemoglobin levels return to normal, people who have had their blood donation status revoked because of low hemoglobin levels are expected to donate 30% less blood than people who have not had their status revoked over the course of the next four to five years (Mast, 2014). Iron deficiency is caused by non-haematological factors other than blood-related problems, and these factors can cause unpleasant symptoms like fatigue, limited physical endurance, trouble managing body temperature, poor cognitive function, and more. It is important to examine the cause of iron deficiency because it is a symptom of a more serious condition rather than a condition in and of itself (Camaschella, 2019). Iron is carried by ferroportin from cells into the bloodstream, where it attaches to transferrin and travels to macrophages.

The most flexible diagnostic test for figuring out iron deficiency is serum ferritin. Pale skin tone and symptoms including dyspnea, vertigo, and cognitive decline might result from central hypoxia. Nonetheless, the pallor could disappear if the anemia is corrected. This study found that whole blood donation failed to complete after three to five minutes in those within the acceptable hemoglobin donation range due to fatigue onset (QoL), confirming that suppressed iron deficiency anemia (IDA) has a severe impact on quality of life (Andro et al., 2013). 200 mg of iron will be lost from a 450 mL blood donation from a donor whose hemoglobin (Hb) level is less than 12.5 g/dl, plus an additional 50 mL for laboratory testing (Haraldstad et al., 2019).

Regular blood donors run the risk of developing iron deficiency (ID), which is characterized by low serum ferritin levels (Tailor et al., 2017). Therefore, it is essential to protect blood donors from these risks. The usefulness of using iron status as the foundation for donor deferral policy, which successfully protects donors from iron insufficiency, has been demonstrated by recent research (Cook et al., 1986). The body's iron reserves can be measured using erythrocyte protoporphyrin, serum ferritin, hemoglobin, and transferrin saturation. Anaemia is commonly caused by an iron shortage. Primary menstrual bleeding and gastrointestinal bleeding are its main causes. For people for whom oral iron is inefficient or contraindicated, iron supplements can be given intravenously or orally (Simon, 2002).

The minimal Hb threshold is intended to prohibit blood donation from donors who are anemic, but it does not stop blood donation from donors who are iron deficient. The main source of life-saving blood for serious medical disorders is regular blood donors. Their primary objectives are to save lives, and blood donations are used in numerous medical settings, such as birth- ing rooms, pediatric departments, adult and neonatal intensive care units, emer- gency rooms, critical care units, operating rooms and surgical units (Mittal et al., 2006). The study sample for this research consists of 300 male and female donors who came to the blood donation facility and were housed in a 300-bed facility. For five months, starting on October 1, 2023, and continuing until February 20, 2024, data will be gathered and categorized weekly. The institutional ethics council and the scientific ethical research committee of Jazan University will provide their ethical approval before this is carried out. Descriptive statistics, frequencies, and percentages were used to evaluate the categorical data in order to show how the characteristics of different laboratory test factors affected the percentage differences amongst the five groups. To compare categorical data, a chi-squared test was used. An unidirectional analysis of variance (ANOVA) was employed to evaluate the effects of different treatments on the iron levels and IBS depletion during the previous four years.

A post hoc test was used to determine which donor groups specifically contributed to the overall significant effect. The differences between the different donor categories were ascertained using an ordinal linear regression analysis. The dependent variable, iron storage reserves, has three levels: normal, decreasing, depleted, and iron serum sideropenia. Ordinal regression is a statistical technique used to determine these levels. This forecast is supported by a number of independent variables. The variable being measured or observed in respect to the response categories' order is referred to as the dependent variable. Conversely, the independent variable may be continuous or categorical. IBM Corp., New York, NY, USA) SPSS version 22.0 was used for the analyses. A statistical significance threshold of $P < 0.05$ was utilized to ascertain the significance (Rigas et al., 2017).

Materials and Methods

The blood bank and laboratory services offered to these elite ABO Rh donor types at Beish G-H and Jazan Nursing and Health Science Hospital University were the subject of a scientific prospective study. These services included medical support provided following the acquisition of both verbal and written consent from the volunteer blood donors at the Beish G. H blood bank donation center. In addition, the study looked at how frequently blood was donated and how habitual blood donors responded to a quick deferral questionnaire when they visited the blood donation facility. The Standing Committee for Scientific Research Jazan University gave its clearance to the work (HAPO-10-Z-001). The period of participant selection was October 2023–February 2024.

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Additionally, blood samples (2.5 mL) containing anticoagulant EDTA are analyzed for hemoglobin using spectrophotometry at the Sysmex XN-550-L-Series automated cell counter. Based on the most recent reality update of the donors' hemoglobin volume and red blood cell indices, acceptance or rejection decisions are made. Serum iron concentrations were measured using sepa-rated serum. A plastic ROCH cuvette was used to dispense 500 μ l of serum. The serum was then put through a BECKMAN COULTER DXC-700-AU Clinical Biochemistry Analyzer (CA, U.S.A.) in accordance with the manufacturer's instructions to assess iron levels.

Study Design

At Beish General Hospital and Jazan University Hospital, a 150-bed tertiary care facility, a scientific prospective study was carried out to look into voluntary whole-blood donors. The study concentrated on the laboratory and blood bank services offered to these elite ABO Rh donor types, together with medical assistance following the Saudi volunteers' verbal and written agreement. The frequency of blood donations and the reactions of frequent blood donors to a brief deferral were also investigated in this study. The Standing Committee for Scientific Research—Jazan University—approved the work (HAPO-10-Z-001). The period of participant selection was October 2023–February 2024.

Donor Selection Criteria

In accordance with the instructions provided in the technical handbook of the Saudi Health Ministry's Directorate General of Health, specific questions on blood donation were posed to the donors. The chosen donors were questioned regarding their educational background, dietary history, medication use, age at the time of the first donation, date of the last donation, number of donations within the previous four years, time intervals between donations, and iron supplementation dosage for those who are aware of its use. Information was also gathered about bodybuilders' usage of hormone therapy, including growth factors and testosterone (where appropriate) (Kirchner & Schlenke, 2000).

Inclusion Criteria

All male and female qualified volunteer donors who had given blood within the previous four years were included in the study. A minimum of 90 days (or three months) had to elapse since the last donation, and the contributors needed to continue their regular practice of giving at least once a year (Vinkenoog et al., 2020).

The study's contributors were then divided into five groups according to the amount of donations they had made: Group I is made up of donors who had made their first donation; Group II is made up of donors who had made two donations; Group III is made up of donors who had made three to four donations; Group IV is made up of donors who had made five to nine donations; and Group V is made up of donors who had not made any donations at all (Skikne et al., 1984).

Exclusion Criteria

- a) All willing donors who did not meet the requirements for safe and healthy donors.
- b) The carrier-S hemoglobin trait, or HBAS.
- b) Every donor who used growth hormone therapy and additional iron during the gem program.

Study Methodology

A total of 300 willing blood donors provided blood samples, with each donor receiving 450 ± 50 mL of whole blood for additional analysis. Following the donation, samples were taken into three mL plain tubes (yellow-top) for this study and into 2.5 mL EDTA (Ethylene diamine tetra acetic acid) tubes for measuring RBC indices once regular volunteer donors gave their consent. The serum was divided, put into simple vitrines, and kept at -20°C to measure the amount of iron, the total iron binding capacity, and the accumulation of donor ferritin (Kaynar et al., 2022).

Haemoglobin Estimation

The hemoglobin levels of 300 willing blood donors were estimated. Using the FRESINIUS KABI Compo-lab TM 61346 model (Hamburg, Germany), one blood drop was collected at the donation unit after the donor sterilised their finger by fracturing it with a lancet. Additionally, blood samples (2.5 mL) containing anticoagulant EDTA are measured for hemoglobin using spectrophotometry at the Sysmex XN-550-L-Series automated cell counter. Based on the most recent reality check of the donor's hemoglobin content, the sample is either accepted or rejected. For male donors, the reference hemoglobin level is 13 g/dl to 15.5 g/dl, while for female donors, it is 12.5 g/dl to 14.5 g/dl (Haider et al., 2017).

Red Cell Indices Calculation

MCV (Mean Corpuscular Volume), MCH (Mean Corpuscular Haemoglobin), and MCHC (Mean Corpuscular Haemoglobin Concentration) were three of the haematological tests performed on the study participants' blood samples. Red cell indices were determined with Coulter, Japan's Sysmex XN-550-L-Series instrument. MCV typically ranges between 80 and 100 fl. MCH ranges from 27 to 31 pages. An adult's MCHC ranges from 32 to 36 g/dl.

Assessment of Iron

In this study, 300 frequent blood donors were prospectively enrolled. Based on their clinical histories and physical tests, all of the donors who were chosen were deemed suitable to donate haemoglobin, with the exception of blood donors who were male (13 g/dl to 15.5 g/dl) or female (12.5 g/dl to 14.5 g/dl).

Serum iron concentrations were measured using serum that had been separated. A plastic ROCH cuvette was used to dispense 500 µl of serum, which was then put through a BECKMAN COULTER DXC-700-AU Clinical Biochemistry Analyzer (CA, U.S.A.) in accordance with the manufacturer's instructions to estimate iron levels (World Health Organization, 2014).

Assessment of Ferritin

The study prospectively included all 300 regular blood donors; serum iron storage concentrations were measured using a different serum. Following the manufacturer's instructions, 1000 µl 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.).

Calculation of Total Iron-Binding Capacity (TIBC)

The total iron-binding capacity (TIBC) test is a crucial diagnostic tool for iron deficiency anemias and other illnesses involving iron metabolism. The BECKMAN COULTER DXC-AU 700 software can be used to retrieve the TIBC value as an iron profile result. Transferrin's ability to bind with iron is known as its iron binding capability. The process of assessing blood donor TIBC involves first adding extra Fe³⁺ to the donor trans-ferin's serum.

The formula used to calculate total iron binding capacity (TIBC) was $TIBC (\mu\text{g/dl}) = \text{Transferrin (mg/dl)} \times 1.2521$.

(An additional reference = Transferrin m g/dl \times 0.025) and multiply by 88.4 to convert m g/dl to µmol/l. The transferrin saturation (TSAT-Transferrin saturation) average (65 - 176) was calculated using the following formula, with the reference range values for TIBC being 255 - 450 m g/dl.

(Serum iron/TIBC) \times 100% is TSAT. (Multiply m g/dl by 88.4 to translate to µmol/l).

Five categories were created from the donors: Group I consisted of first-time donors; Group II included repeat donors; and Group III included two donations. - 4 times; Group V, donors who made 10 - 16 donations in the previous four years; and Group IV, donors who made 5 - 9 donations. Serum ferritin levels were classified as follows: depleted at less than 12 ng/ml, reduced at 15–24 ng/ml, normal or replete at 24–337 ng/ml, and raised at more than 300 µg/dl. Serum ferritin levels less than 12 ng/ml were deemed indicative of iron deficiency anemia (Brislin et al., 2017).

The study prospectively included all 300 regular blood donors; serum iron storage concentrations were measured using a different serum. Following the manufacturer's instructions, 1000 µl 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.). IBM Corp., New York, NY, USA) SPSS version 22.0 was used for the analyses. A statistical significance threshold of $P < 0.05$ was utilized to ascertain the significance.

Results

Of the 300 donors involved in this study, 285 (95%) were men and 15 (5%) were women (Table 1 and Figure 1). The women who took part ranged in age from 22 to 48. The male patients ranged in age from 17 to 51, with a mean age of 33 ± 8 for both groups overall. Male donors make up the majority of the study group (95%). Figure 1 and Table 1.

Table 1. shows the breakdown of the study sample's male and female participants.

Gender	Frequenc y	Percen t
Female	15	5.0
Male	285	95.0
Total	300	100.0

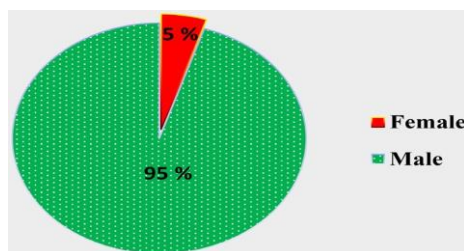


Figure 1: Gender distribution of volunteer blood donors, N = 30

A total of 264 donors (88%) had given blood at least once in the preceding three years, whereas 36 donors (12%) were first-time blood donors with no prior blood donation experience. Based on the number of prior donations made during the previous four years, the population under research was split into five groups (Table 2). The blood donors in Group II contributed on an average of one to two times a year and were considered regular donors. This group of donors consists of 58 donors, or 19.3% of all donors. According to the results, contributors in Group III have contributed three or four times. This category consists of 79 donors, or 26.3% of all donors. Group IV donors have contributed five to nine times.

Group IV donors have contributed five to nine times. This category comprises 60 donors, which represents 20.0% of all donors. Donors who have contributed between 10 and 16 times are in Group V. This category has 67 donors, accounting for 22.3% of all donors. The group I donors (Table 3) have somewhat greater MCV and MCH values than the other groups, indicating that their red blood cells are larger and contain more hemoglobin. Additionally, this group has relatively larger quantities of iron and ferritin. Group II: Blood parameters from Group I are slightly different in the donors; MCV and MCH values are slightly lower, while the concentrations of iron and ferritin are slightly higher.

Table 2. Donors are divided into age groups.

Groups	Number of Donations	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.

Group III's blood measurements exhibit trends that are comparable to those of Group II, with minor variations in the amounts of iron, ferritin, MCV, and MCH. In the meantime, Group IV's blood values show a modest drop in the amounts of iron, ferritin, MCV, and MCH when compared to earlier groups. The blood parameters for Group V, as shown in Table 3 and Figures 2–7, additional declines in MCV, MCH, iron, and ferritin concentrations are seen. This suggests that frequent blood donation may have an impact on these parameters, particularly ferritin.

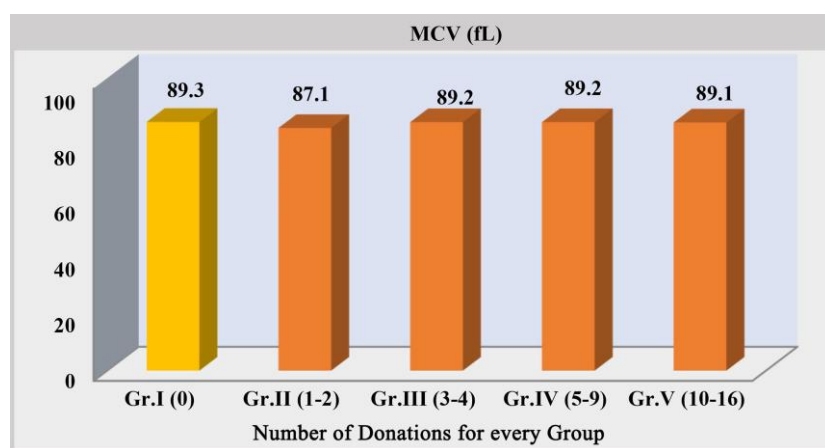


Figure 2. MCV levels among blood donors based on frequency of donation.

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

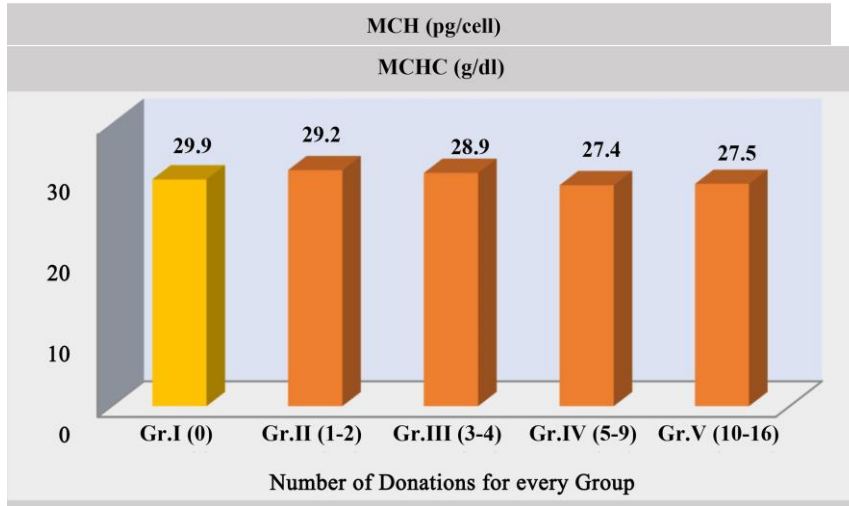


Figure 4. MCHC concentrations of blood donors according to the frequency of donation.

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

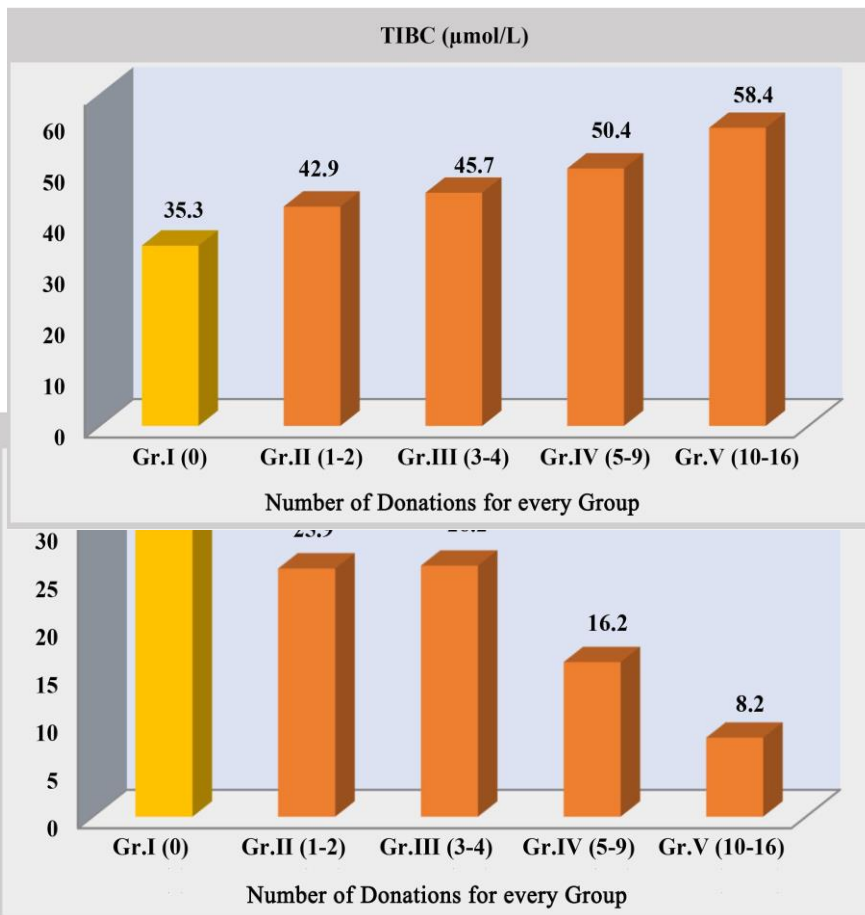


Figure 6. TIBC concentrations of blood donors according to frequency of donation.

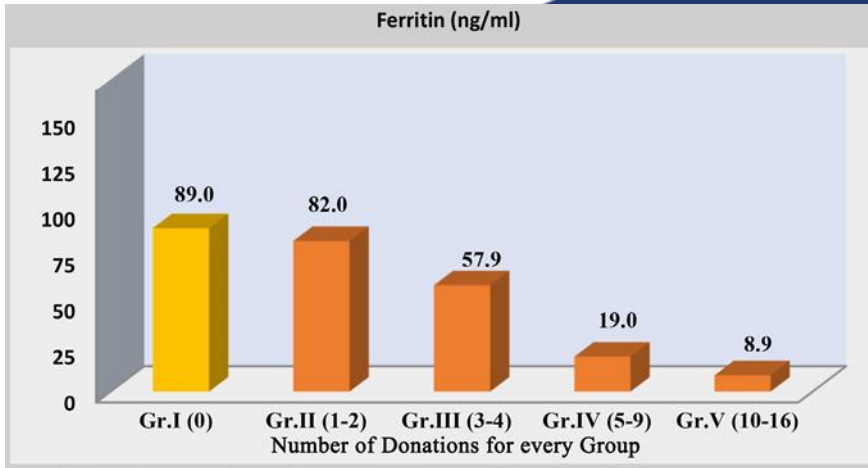


Figure 7. Ferritin concentrations of blood donors according to frequency of donation.

Based on the frequency of contributions, the data shown in (Table 4) show the p-values indicating the differences between the means of MCV, MCH, iron, TIBC, and ferritin levels between donor groups and the control group (Group I). This is how it is interpreted. When the p-value is less than 0.0125, statistically significant differences are shown in cells with the symbol "*." The Bonferroni procedure is used to alter this significant level while taking into account several 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					
	MC V	MC H	MCH C	Iron	TIB C	Ferriti n
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. After dividing the significance level of 0.05 by 4, we performed four comparisons between the control group I and groups II and V.

In Group I (0 donations), the majority of donors (91.7%) had normal iron storage, while 5.6% (2/36) of donors had reduced iron stores. (Table 4).

The percentage of donors with normal iron storage steadily declines as the number of donations rises (Groups II to V), whereas the percentage of donors with decreased, depleted, and severely deficient iron stores increases.

Remarkably, in Group V (10 - 16 donations), the majority of donors show evidence of iron shortage, with a sizable share (77.6%) classified as severely deficient. In contrast, just 1.5% of donors have normal iron storage.

All things considered, the table shows a relationship between the number of blood donations and the condition of iron storage, with frequent donors showing a higher likelihood of iron insufficiency.

The study employed cumulative odds ordinal logistic regression with proportional odds, which uses cumulative categories, to analyze the relationship between the dependent variable—which indicates iron deficiency—and the four levels of ferritin concentration: normal > 24 ng/ml, reduced iron (15 - 24 ng/ml), depleted iron (< 12 ng/ml), and sidero-penia (< 10.70 umol/l). Table 5 Demonstrates that donors are 0.82 (95% CI, -0.79 to -0.86) times less likely to be in a higher category of "Ferritin concentration" if their iron level increases by one unit. This indicates that the likelihood of the donors falling into the typical category is 18%. $P < 0.001$, Wald $\chi^2(1) = 80.61$.

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	Std. Error	Wald	df	Sig	Exp, 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	80.6	1	0.000 *	0.82	0.79	0.86
TIBC	0.03	0.01	6.07	1	0.014 *	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
Gr. II (1 - 2)	0.34	0.84	0.16	1	0.687	1.40	0.27	7.30
Gr. III (3 - 4)	1.47	0.82	3.20	1	0.074	4.34	0.87	21.67
Gr. IV (5 - 9)	2.68	0.83	10.4	2	0.001 *	14.56	2.86	74.05
Gr. V (10 - 16)	2.75	0.91	9.23	1	0.002 *	15.69	2.66	92.62

In contrast, people with increasing "TIBC" levels appear to be 1.03 (95% CI, 1.01 to 1.05) times more likely to fall into the higher category of the ordinal outcome, or sideropenia, than people with decreasing TIBC levels. This difference is statistically significant, as indicated by Wald $\chi^2(1) = 6.07$, $P = 0.0014$.

Discussion and conclusion

According to the average age of the study group, 300 people who frequently donate blood were evaluated for the presence of iron deficiency anemia. There were 15 (5%) females and 285 (95%) males among them. Male blood donors range in age from 17 to 51 years, while female donors range in age from 22 to 48 years. The average age of both genders is 33 \pm 8 years. The lack of statistical significance in the result implies that age and gender had no discernible influence on the onset of iron deficiency anemia.

The study population was divided into five groups according to how frequently they had donated blood in the previous four years. The blood donors in Group 1, also referred to as the Control group, had never donated blood before and were giving it away; in contrast, the blood donors in Groups 2 through 5 donated blood frequently, with an average frequency ranging from two to sixteen times in the previous four years. Significant differences were observed in the average serum ferritin levels among the five groups (G-1 to G-5), both for males and females (Scott, 1982).

Numerous investigations have demonstrated that long-term blood donors frequently have chronic iron deficiency (Finch et al., 1977). A condition known as iron deficiency occurs when the body's normal level of iron falls below average. The Hemoglobin (Hb) test is the screening tool currently used to assess a blood donor's eligibility for donation. The minimal Hb threshold is intended to prevent blood donors with anemia from being drawn, however it does not stop blood donors with iron deficiency from being drawn (Alexander et al., 2000).

According to this study, 35% of those who routinely give blood suffer with sideropenia, or iron-deficient anemia.

Group 1 (Control group) had iron and ferritin concentrations within the normal range, however the other four groups (G-2 to G-5) had lower concentrations. It is well known that frequent blood donations lower iron reserves (Sideropenic Aneamia). Published studies have employed numerous indices to diagnose iron deficiency and depletion in blood donors (Pedersen & Morling, 1978).

The results of the study show that ferritin concentrations significantly decreased as the frequency of contributions increased. The results are consistent with other researches' findings and conclusions. The amount of iron that is able to bind to unsaturated transferrin is measured by the total iron-binding capacity (TIBC). However, because its values remain constant until iron stores are all exhausted, it has been suggested that the haematological characteristics show that (TIBC) is not a reliable indication of an early iron deficiency (Milman, 1996). As donations grew, this study discovered statistically significant alterations in (TIBC).

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

	ng/ml	ng/ml	ng/ml	(sideropenia) < 10.7 umol/L	n (%)
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%)

After just five contributions, habitual blood donors' average * ferritin levels significantly decreased. This implies that serum ferritin testing must to be done sooner rather than later, ideally following three donors.

Proportionately, the degree of iron reserve (ferritin) depletion rose in each group as the number of donations increased. In particular, there was a rise of 3.8% for group III, a 5% increase for group IV, and a 9% increase for group V. These results are consistent with earlier research (Tardtong et al., 2000).

This study's findings indicate a noteworthy prevalence of decreased iron storage, especially in those who regularly donate blood even when they are not eligible to do so. It would be safer, sufficient, and more repeatable to donate blood if methods were put in place that more accurately show iron status, such as ferritin level and serum iron concentration. This would benefit all donors (Cançado et al., 2001).

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