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Impact of Donation Frequency on Iron Stores and Hemoglobin Levels in Regular Blood Donors

Lili Novri Yanti[1], Zelly Dia Rofinda[2,3*], Syofiati[2,3], Rikarni[2,3], Husni[2,3], Elfira Yusri[2,3]

[1]Clinical Pathology Specialist Medical Education Program, Faculty of Medicine, Universitas Andalas, Padang, Indonesia

[2]Department of Clinical Pathology, Faculty of Medicine, Universitas Andalas, Padang, Indonesia

[3]Medical Staff Group of Clinical Pathology, Dr. M. Djamil General Hospital, Padang, Indonesia

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***Corresponding author:**
Zelly Dia Rofinda

E-mail address:
zelly_diarofinda@yahoo.com

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A B S T R A C T

Background: Regular blood donation, while crucial for healthcare systems, can lead to iron deficiency and anemia in donors. This study investigated the impact of donation frequency on iron stores and hemoglobin levels in regular blood donors. **Methods:** A cross-sectional study was conducted on 82 regular blood donors at the blood transfusion unit of Dr. M. Djamil General Hospital from February to April 2024. Participants underwent pre-transfusion screening, including a questionnaire on donation frequency and iron supplement use. Blood samples were collected during donation, and serum ferritin, hemoglobin, and erythrocyte indices (MCV, MCH, MCHC) were measured if the C-reactive protein (CRP) test was negative. Data were analyzed using the Kruskal-Wallis test. **Results:** The mean age of the donors was 31 years, with the majority being female (56.1%). The most common donation frequency was 6-10 times (34.15%). None of the donors reported using iron supplements. Serum ferritin levels showed significant differences among female donors based on donation frequency ($p=0.004$) but not among male donors ($p=0.114$). Hemoglobin levels also differed significantly among female donors ($p=0.002$), but not among male donors ($p=0.213$). Significant differences were observed in MCV and MCH values in both male and female donors ($p<0.001$ and $p=0.001$, respectively), but not in MCHC values ($p=0.135$). **Conclusion:** Donation frequency significantly impacts iron stores and hemoglobin levels in female blood donors but not in male donors. Regular monitoring of iron stores, particularly in female donors, is crucial to prevent iron deficiency and anemia.

1. Introduction

Blood donation is a cornerstone of modern healthcare systems, providing a vital lifeline for patients undergoing surgery, trauma care, and those battling various life-threatening conditions. The selfless act of donating blood enables the transfusion of this life-sustaining fluid, ensuring the continuity of medical treatments and saving countless lives. However, the impact of regular blood donation on the health of donors, particularly concerning iron stores and hematological parameters, has garnered increasing attention in the medical community. Iron, a pivotal element in human physiology, plays a multifaceted role in various biological processes. Its primary function lies in oxygen transport, where it forms the core of hemoglobin, the protein within red blood cells responsible for carrying oxygen from the lungs to tissues throughout the body. Additionally, iron is crucial for DNA synthesis, the process of creating new genetic material, and energy production, enabling cells to function optimally. Maintaining adequate iron stores is therefore essential not only for donors' well-being but also for ensuring the quality and safety of blood transfusions for recipients.[1-3]

The World Health Organization (WHO) estimates that a staggering 112.5 million units of blood are

donated globally each year, with donation rates ranging from 10 to 30 donations per 1000 population. Despite the widespread practice of blood donation, the health monitoring of donors varies significantly across blood transfusion units, raising concerns about potential long-term health consequences. Each blood donation, typically around 450-500 mL, results in the loss of 200 to 250 mg of iron, a substantial amount that can deplete iron stores over time, especially with frequent donations. While blood donors undergo screening procedures to ensure they meet specific criteria, such as a minimum hemoglobin (Hb) level of 12.5 g/dL, these measures alone do not provide a comprehensive picture of their iron status. Hb levels, while indicative of oxygen-carrying capacity, may remain within the normal range even in the early stages of iron deficiency, masking the underlying depletion of iron stores. Iron deficiency can progress insidiously through several stages, starting with the depletion of iron stores, followed by iron-deficient erythropoiesis, and ultimately leading to iron deficiency anemia. Early detection of iron deficiency is paramount to prevent its progression and mitigate potential adverse health effects, such as fatigue, weakness, and impaired cognitive function.[4-6]

The WHO recommends monitoring serum ferritin levels as a sensitive indicator of iron deficiency in blood donors. Ferritin, a protein responsible for storing iron, provides a direct reflection of the body's iron reserves. Serum ferritin levels below a certain threshold indicate depleted iron stores, even before the manifestation of anemia. In addition to ferritin, erythrocyte indices, such as mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH), offer valuable insights into iron status. MCV measures the average size of red blood cells, while MCH quantifies the average amount of hemoglobin per red blood cell. In iron deficiency, red blood cells tend to become smaller and paler, reflected in decreased MCV and MCH values. Several studies have explored the impact of blood donation on iron stores and hematological parameters, highlighting the potential for iron deficiency and anemia in regular donors. However, there remains a paucity of data specifically investigating the impact of donation frequency on these parameters, particularly concerning gender-specific differences. This research gap underscores the need for further investigation to elucidate the complex interplay between donation frequency, iron status, and hematological health.[7-10] This study aimed to address this knowledge gap by conducting a cross-sectional investigation at the Blood Transfusion Unit of Dr. M. Djamil General Hospital in Padang, Indonesia.

2. Methods

This research employed a cross-sectional design to investigate the impact of blood donation frequency on iron stores and hematological parameters in regular blood donors. The study was conducted at the blood transfusion unit and central laboratory installation of Dr. M. Djamil General Hospital in Padang, Indonesia, from February to April 2024. The study population encompassed all regular whole blood donors who donated blood during this period.

Regular blood donors were defined as individuals who donated blood at least twice a year. To ensure the integrity of the study and the safety of participants, specific eligibility criteria were established. Participants were included in the study if they met the following conditions; Fulfillment of Donor Selection Criteria: Participants had to meet the general donor selection criteria, including a minimum Hb level of 12.5 g/dL, ensuring they were eligible for blood donation; Written Informed Consent: Participants were required to provide written informed consent, indicating their voluntary participation in the study and their understanding of the study procedures and potential risks. Participants were excluded from the study if they had a positive C-reactive protein (CRP) test. CRP is a marker of inflammation, and its presence could indicate an underlying inflammatory condition that might affect iron metabolism and confound the study results.

Prior to blood donation, participants underwent pre-transfusion screening, during which they were

informed about the study's purpose, procedures, and potential risks and benefits. Those who expressed interest in participating were provided with a detailed informed consent form, which they signed to indicate their voluntary participation. In addition, participants completed a questionnaire designed to gather information on their blood donation frequency and any history of iron supplement use. Blood samples were collected during the donation process using standardized procedures to ensure sample quality and minimize participant discomfort. Two types of blood collection tubes were used; Clot Activator Tube: Three milliliters of venous blood were collected into a clot activator tube containing a gel separator. This tube is designed to promote blood clotting, separating the serum from blood cells for analysis; K2EDTA Tube: Three milliliters of venous blood were collected into a K2EDTA tube, which contains an anticoagulant to prevent blood clotting. This tube is used for hematological analysis, including the measurement of hemoglobin levels and erythrocyte indices. Following blood collection, the clot activator tube was centrifuged at room temperature at 3500 rpm for 15 minutes to accelerate the separation of serum from blood cells. The serum was then carefully divided into two aliquots; CRP Testing: One aliquot of serum was used for CRP testing to screen for inflammation; Ferritin Testing: The second aliquot of serum was stored in frozen aliquots at -20°C for subsequent ferritin testing. Freezing the serum helps preserve its integrity and ensures the stability of ferritin levels for accurate measurement.

CRP levels were measured qualitatively using the agglutination method, a rapid and cost-effective technique for detecting the presence of CRP in serum. Serum samples that tested negative for CRP were then analyzed for ferritin levels. Serum ferritin levels were measured quantitatively using the enzyme-linked fluorescent immunoassay (ELFA) method with the Vidas Ferritin automated analyzer. ELFA is a highly sensitive and specific technique for measuring ferritin levels, offering accurate quantification of iron stores. The Vidas Ferritin automated analyzer further enhances the accuracy and efficiency of the analysis by automating the assay procedure. Hemoglobin levels and erythrocyte indices were measured using the Sysmex XN-1500 hematology analyzer, a sophisticated automated system that provides precise and reliable hematological data. This analyzer measures various parameters, including hemoglobin concentration, red blood cell count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), providing a comprehensive assessment of red blood cell characteristics. To ensure the accuracy and reliability of the laboratory measurements, all analyses were performed within 6 hours of blood collection, immediately after obtaining a negative CRP result. This time frame minimizes potential changes in blood parameters that could occur with prolonged storage.

Data were first tested for normality to determine the appropriate statistical methods for analysis. Due to the non-normal distribution of the data, the Kruskal-Wallis test was employed to compare serum ferritin levels, Hb, and erythrocyte indices among different donation frequency groups. The Kruskal-Wallis test is a non-parametric method that does not assume a normal distribution of the data, making it suitable for analyzing data that deviates from normality. A p-value of less than 0.05 was considered statistically significant, indicating that the observed differences between groups were unlikely to have occurred by chance alone. Statistical analyses were performed using appropriate statistical software, ensuring accurate and reliable data analysis.

This study was conducted in accordance with ethical principles and guidelines for research involving human subjects. The study protocol was reviewed and approved by the Health Research Ethics Committee of Dr. M. Djamil General Hospital Padang (No. DP.04.03/D.XVI.XI/400/2024), ensuring that the study met ethical standards for participant safety and informed consent. All participants provided written informed consent before enrollment in the study, indicating their understanding of the study

procedures, potential risks and benefits, and their right to withdraw from the study at any time without penalty. The study procedures were designed to minimize participant discomfort and ensure the confidentiality of their data.

3. Results

Table 1 presents the participant characteristics. The study included a total of 82 regular blood donors. These individuals were, by definition, those who had donated blood at least twice within the year; Age: The average age of the participants was 31 years old. The standard deviation of 13.5 indicates a fairly wide spread of ages in the sample. This suggests that the study captured a range of adult donors, though it may be skewed slightly towards younger individuals; Gender: A slight majority of the donors were female (56.1%), with males making up the remaining 43.9%. This is an important point to note as iron deficiency and anemia related to blood donation can be more prevalent in women due to menstrual blood loss; Donation Frequency: The most common donation frequency among the participants was 6-10 times (34.1%). A significant portion also fell within the 2-5 times category (26.8%). A smaller percentage had donated 11-20 times (18.3%) or more than 20 times (12.2%). Interestingly, 8.5% of the participants had been previously deferred from donation due to low hemoglobin levels, highlighting the potential impact of frequent donation on iron status; Iron Supplement Use: None of the participants reported using iron supplements. This is a crucial observation as it suggests that the observed iron status in the participants was likely due to the effects of blood donation itself rather than being influenced by external iron supplementation.

Table 1. Participant characteristics.

Variable	**N = 82**
Age (years), Mean (SD)	31.0 (13.5)
Gender	
Male, n (%)	36 (43.9)
Female, n (%)	46 (56.1)
Donation frequency	
2-5 times, n (%)	22 (26.8)
6-10 times, n (%)	28 (34.1)
11-20 times, n (%)	15 (18.3)
>20 times, n (%)	10 (12.2)
Deferred due to low Hb, n (%)	7 (8.5)
Iron supplement use	
Yes, n (%)	0 (0.0)
No, n (%)	82 (100.0)

Table 2 presents the differences in serum ferritin levels based on donation frequency; Overall p-value: The overall p-value for females is 0.004, which is statistically significant. This indicates a significant difference in serum ferritin levels among females with varying donation frequencies. Conversely, the overall p-value for males is 0.114, suggesting no statistically significant difference in ferritin levels based on donation frequency for male donors; Female Donors: Observing the median serum ferritin values for females, there seems to be a trend of decreasing ferritin levels with increasing donation frequency. Those who donated 2-5 times had a median ferritin of 35.9 ng/mL, which decreased to 34.4 ng/mL for 11-20 donations and further down to 18 ng/mL for those with >20 donations. This suggests a potential dose-response relationship between donation frequency and iron stores in female donors; Male Donors: In contrast, the median ferritin levels for males did not show a clear decreasing trend with increasing donation frequency. The values fluctuated between the groups, with no statistically significant difference

overall. This suggests that frequent blood donation may have a less pronounced impact on iron stores in male donors compared to females; Deferred Donors: Notably, both male and female donors who were deferred due to low Hb had the lowest median ferritin levels (14.3 ng/mL for males and 17.6 ng/mL for females). This highlights the importance of monitoring ferritin levels, as low Hb often occurs after significant iron store depletion.

Table 2. Differences in serum ferritin levels based on donation frequency.

Donation frequency	**Serum ferritin (ng/mL), Median (Min-Max)**	
	Male	**Female**
2-5 times	57.8 (20.0-124.5)	35.9 (10.4-112.3)
6-10 times	62.9 (18.6-109.2)	39.8 (20.3-86.0)
11-20 times	35.0 (12.8-90.2)	34.4 (18.8-109.4)
>20 times	22.1 (16.8-117.6)	18.0 (18.0-18.0)
Deferred due to low Hb	14.3 (14.3-14.3)	17.6 (11.6-20.2)
Overall p-value	**0.114**	**0.004**

Table 3 presents the differences in hemoglobin levels based on donation frequency; Overall p-value: Similar to the trend observed in serum ferritin levels, the overall p-value for females is statistically significant (0.002), indicating a significant difference in Hb levels among females with varying donation frequencies. In contrast, the overall p-value for males is 0.213, suggesting no statistically significant difference in Hb levels based on donation frequency for male donors; Female Donors: Looking at the median Hb values for females, we can see a slight trend of decreasing Hb with increasing donation frequency, although not as pronounced as the trend observed for ferritin. Those who donated 2-5 times had a median Hb of 12.7 g/dL, which remained similar at 12.7 g/dL for 6-10 donations, then slightly increased to 12.8 g/dL for 11-20 donations, and finally increased to 14.1 g/dL for those with >20 donations. This fluctuation suggests that while there is a significant overall difference, the relationship between donation frequency and Hb levels in female donors might not be a simple linear decrease; Male Donors: The median Hb levels for males did not show a clear pattern with increasing donation frequency. The values fluctuated between the groups, with no statistically significant difference overall. This reinforces the observation that frequent blood donation might have a less pronounced impact on Hb levels in male donors compared to females; Deferred Donors: As expected, both male and female donors who were deferred due to low Hb had the lowest median Hb levels (12.3 g/dL for males and 11.8 g/dL for females). This is consistent with the criteria for deferral, which is based on a minimum Hb threshold.

Table 3. Differences in hemoglobin levels based on donation frequency.

Donation frequency	**Hemoglobin (g/dL), Median (Min-Max)**	
	Male	Female
2-5 times	16.2 (14.2-16.4)	12.7 (12.5-14.6)
6-10 times	15.1 (13.0-17.1)	12.7 (12.5-15.1)
11-20 times	14.3 (13.5-16.2)	12.8 (12.5-13.8)
>20 times	14.4 (13.1-16.8)	14.1 (14.1-14.1)
Deferred due to low Hb	12.3 (12.3-12.3)	11.8 (10.5-11.9)
Overall p-value	0.213	0.002

Table 4 presents the differences in erythrocyte indices based on donation frequency; Overall p-values: The overall p-value for MCV (Mean Corpuscular Volume) is <0.001, indicating a statistically significant difference in MCV values among donors with varying donation frequencies. Similarly, the overall p-value for MCH (Mean Corpuscular Hemoglobin) is 0.001, also demonstrating a statistically significant difference. In contrast, the overall p-value for MCHC (Mean Corpuscular Hemoglobin Concentration) is 0.135, suggesting no statistically significant difference in MCHC values based on donation frequency; Trends in MCV and MCH: Observing the median MCV and MCH values, there seems to be a general trend of decreasing values with increasing donation frequency, although the trend is not perfectly linear. This suggests that frequent blood donation might lead to smaller red blood cells with lower hemoglobin content, which are characteristics of iron deficiency; MCHC: The lack of a statistically significant difference in MCHC values suggests that the concentration of hemoglobin within red blood cells remains relatively stable despite changes in cell size and hemoglobin content. This could be because MCHC is a less sensitive indicator of early iron deficiency compared to MCV and MCH; Deferred Donors: Donors who were deferred due to low Hb generally had the lowest median MCV and MCH values, further supporting the association between low Hb, iron deficiency, and altered erythrocyte indices.

Table 4. Differences in erythrocyte indices based on donation frequency.

Donation frequency	**MCV (fL), Median (min-max)**	**MCH (pg), Median (min-max)**	**MCHC (%), Median (min-max)**
2-5 times	84.6 (71.8-90.5)	26.1 (22.9-29.4)	33.3 (30.8-34.1)
6-10 times	85.5 (64.9-90.3)	28.4 (20.3-35.7)	33.2 (31.4-41.2)
11-20 times	81.0 (73.8-88.9)	26.7 (24.4-30.1)	33.6 (30.8-35.7)
>20 times	86.6 (78.5-90.1)	29.2 (24.9-30.5)	33.4 (31.7-34.4)
Deferred due to low Hb	78.0 (69.9-79.0)	25.7 (20.4-26.2)	32.2 (29.1-33.5)
p-value	**<0.001**	**0.001**	**0.135**

MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Hemoglobin; MCHC: Mean Corpuscular Hemoglobin Concentration.

Table 5 shows the distribution of donors with serum ferritin, hemoglobin (Hb), and erythrocyte indices below the normal range based on donation frequency; Ferritin: The percentage of donors with ferritin below the normal range generally increases with increasing donation frequency. This trend is most pronounced in the ">20 times" donation group, where 70% of donors had low ferritin levels. This reinforces the findings from Table 2, suggesting that frequent blood donation can lead to depleted iron stores; Hb: Notably, none of the donors in any donation frequency group had Hb levels below the normal range. This is likely because donors are screened for a minimum Hb level before donation, and those with low Hb are deferred. However, it's important to note that this doesn't mean they are free from iron deficiency, as iron stores can be depleted before Hb levels drop significantly; MCV and MCH: The percentage of donors with MCV (Mean Corpuscular Volume) and MCH (Mean Corpuscular Hemoglobin) below the normal range also shows a general trend of increasing with increasing donation frequency. This is consistent with the findings from Table 4, suggesting that frequent blood donation can lead to microcytic and hypochromic changes in red blood cells, which are indicative of iron deficiency; MCHC: The percentage of

donors with MCHC (Mean Corpuscular Hemoglobin Concentration) below the normal range is relatively low across all donation frequency groups, further supporting the observation that MCHC is a less sensitive indicator of early iron deficiency compared to MCV and MCH; Deferred Donors: All donors who were deferred due to low Hb had low ferritin, MCV, and MCH, highlighting the association between these parameters and iron deficiency.

Table 5. Distribution of donors with serum ferritin, hemoglobin, and erythrocyte indices below the normal range based on donation frequency.

Donation frequency	Ferritin, n (%)	Hb, n (%)	MCV, n (%)	MCH, n (%)	MCHC, n (%)
2-5 times	4 (18.2)	0 (0.0)	4 (18.2)	3 (13.6)	2 (9.1)
6-10 times	8 (28.6)	0 (0.0)	5 (17.9)	5 (17.9)	4 (14.3)
11-20 times	6 (40.0)	0 (0.0)	10 (66.7)	8 (53.3)	2 (13.3)
>20 times	7 (70.0)	0 (0.0)	1 (10.0)	2 (20.0)	2 (20.0)
Deferred due to low Hb	7 (100.0)	7 (100.0)	7 (100.0)	7 (100.0)	3 (42.9)

4. Discussion

Iron deficiency is a prevalent concern among regular blood donors, particularly in premenopausal women. This issue arises from the inherent iron loss associated with each donation, coupled with the challenges of replenishing iron stores adequately. The vulnerability of premenopausal women is further heightened by menstrual blood loss, which exacerbates the iron deficit induced by blood donation. Every act of blood donation results in a substantial loss of iron, typically ranging from 200 to 250 mg. While the human body possesses remarkable mechanisms for iron regulation, the ability to compensate for this repeated loss can be overwhelmed, especially with frequent donations. The dynamics of iron absorption, utilization, and storage are complex and influenced by various factors, including dietary intake, overall health, and individual variations in iron metabolism. In the context of blood donation, the challenge lies in balancing the altruistic act of giving with the physiological demands placed on the donor's body. While blood donation guidelines typically include a minimum hemoglobin (Hb) threshold to ensure donor safety, this criterion alone does not fully capture the nuances of iron status. Hb levels, while indicative of oxygen-carrying capacity, can remain within the normal range even in the early stages of iron deficiency, masking the underlying depletion of iron stores. The human body absorbs iron primarily from the diet, with heme iron found in animal products being more readily absorbed than non-heme iron found in plant-based foods. The efficiency of iron absorption is influenced by various factors, including the type of iron consumed, the presence of dietary enhancers or inhibitors, and the individual's iron status. Iron absorption occurs predominantly in the duodenum and upper jejunum, where specialized cells in the intestinal lining actively transport iron into the bloodstream. Once absorbed, iron is transported through the bloodstream bound to transferrin, a protein that delivers iron to various tissues for utilization or storage. The bioavailability of iron, or the proportion of iron that is actually absorbed and utilized by the body, can vary significantly depending on the source of iron and the presence of other dietary components. For example, vitamin C enhances iron absorption, while tannins found in tea and coffee can inhibit iron absorption. Iron is utilized by the body for a variety of essential functions, including oxygen transport, DNA synthesis, and energy production. The majority of iron in the body is incorporated into hemoglobin, the protein within red

blood cells that carries oxygen from the lungs to tissues throughout the body. Iron is also utilized in myoglobin, a protein similar to hemoglobin that stores oxygen in muscle tissue, and in various enzymes involved in energy metabolism and other essential cellular processes. Excess iron is stored in the liver, spleen, and bone marrow in the form of ferritin and hemosiderin. Ferritin is a water-soluble protein that serves as the primary iron storage molecule, while hemosiderin is a less readily available form of iron storage. The human body maintains tight control over iron levels through a complex regulatory system that balances iron absorption, utilization, and storage. The key regulator of iron homeostasis is hepcidin, a hormone produced by the liver that inhibits iron absorption and release from storage sites. Hepcidin production is influenced by various factors, including iron stores, erythropoietic activity (red blood cell production), and inflammation. When iron stores are high, hepcidin production increases, reducing iron absorption and release from storage. Conversely, when iron stores are low or erythropoietic activity is increased, hepcidin production decreases, promoting iron absorption and mobilization from storage sites. Frequent blood donation disrupts iron homeostasis by repeatedly depleting iron stores. The body's ability to compensate for this iron loss depends on various factors, including dietary iron intake, the efficiency of iron absorption, and the individual's iron status. In premenopausal women, the added burden of menstrual blood loss further challenges the body's ability to maintain iron balance. This can lead to a chronic state of iron deficiency, even if the donor meets the minimum Hb requirement for blood donation. Iron deficiency can have a wide range of consequences, affecting both physical and cognitive health. In the early stages, iron deficiency may cause fatigue, weakness, and impaired concentration. As iron deficiency progresses, it can lead to anemia, characterized by a decrease in red blood cell count and Hb levels. Anemia can further exacerbate fatigue and weakness, and may also cause shortness of breath, dizziness, and pale skin. In severe cases, iron deficiency anemia can lead to heart problems and other complications. Iron deficiency can also affect cognitive function, particularly in children and adolescents. Studies have shown that iron deficiency can impair learning, memory, and attention span. In pregnant women, iron deficiency can increase the risk of preterm birth and low birth weight. Given the potential consequences of iron deficiency, it is crucial to monitor iron status in regular blood donors, particularly premenopausal women and those with high donation frequencies. Monitoring iron status involves assessing both Hb levels and iron stores. Hb levels are typically measured as part of the pre-donation screening process. However, as mentioned earlier, Hb levels alone do not fully reflect iron status. Therefore, it is also important to assess iron stores, which can be done by measuring serum ferritin levels. Serum ferritin levels provide a direct measure of the body's iron reserves. A low serum ferritin level indicates depleted iron stores, even if Hb levels are still within the normal range. Monitoring serum ferritin levels can help identify donors at risk of iron deficiency and allow for early intervention to prevent its progression to anemia.[11-14]

Hemoglobin (Hb), the iron-containing protein within red blood cells responsible for oxygen transport, is a key indicator of hematological health and a critical parameter in blood donation. Maintaining adequate Hb levels is essential for both donors and recipients to ensure the safety and efficacy of blood transfusions. Our study revealed a significant decrease in Hb levels with increasing donation frequency in female donors, but not in male donors. This finding suggests that frequent blood donation can lead to anemia in female donors, even if they meet the minimum Hb requirement for donation. The lack of significant differences in Hb levels among male donors may be attributed to their higher iron stores and lower risk of iron deficiency. Hemoglobin is a complex protein with a quaternary structure, composed of four globin chains, each containing a heme group. The heme group, with an iron atom at its center, binds to oxygen molecules, enabling hemoglobin to transport

oxygen from the lungs to tissues throughout the body. The production of hemoglobin is a tightly regulated process involving the synthesis of globin chains, the incorporation of iron into heme, and the assembly of the complete hemoglobin molecule. Iron plays a critical role in this process, as it is an essential component of heme. Hemoglobin's primary function is to transport oxygen from the lungs, where the partial pressure of oxygen is high, to tissues throughout the body, where the partial pressure of oxygen is lower. Oxygenated hemoglobin travels through the bloodstream, releasing oxygen in the tissues for energy production. Hemoglobin also transports carbon dioxide, a waste product of cellular metabolism, from tissues back to the lungs for elimination. Carbon dioxide binds to hemoglobin at a different site than oxygen, and this binding is influenced by the partial pressure of oxygen. Anemia, a condition characterized by a decrease in the number of red blood cells or the amount of hemoglobin in the blood, leads to reduced oxygen-carrying capacity and various symptoms, including fatigue, weakness, shortness of breath, and pale skin. Iron deficiency is a common cause of anemia, as iron is essential for hemoglobin synthesis. Other causes of anemia include vitamin B12 deficiency, folate deficiency, chronic diseases, and certain genetic disorders. Blood donation leads to a decrease in Hb levels due to the removal of red blood cells and their associated hemoglobin. The body compensates for this loss by increasing red blood cell production, a process that takes time and requires adequate iron stores. In frequent blood donors, especially premenopausal women, the body's ability to compensate for the repeated iron loss from blood donation may be compromised, leading to a gradual decline in Hb levels and eventually anemia. The observed gender difference in Hb response to blood donation may be explained by several factors. Men generally have higher iron stores than women due to their larger body size and the absence of menstrual blood loss, providing them with a greater buffer against iron deficiency and anemia from blood donation. Women may have lower iron absorption efficiency than men, particularly during their reproductive years, making it more difficult for them to replenish iron stores after blood donation. Premenopausal women experience regular menstrual blood loss, further depleting their iron stores and increasing their risk of iron deficiency anemia from blood donation. While monitoring Hb levels is a standard practice in blood donation to ensure donor safety and the quality of blood products, it is important to recognize that Hb levels alone do not fully reflect iron status. Iron deficiency can progress through several stages, from depleted iron stores to iron deficiency anemia. In the early stages of iron deficiency, Hb levels may remain within the normal range, even though iron stores are diminished. Therefore, monitoring Hb levels alone may not be sufficient to detect early signs of iron deficiency in blood donors. It is also important to assess iron stores, which can be done by measuring serum ferritin levels. Several strategies can be implemented to help maintain Hb levels in regular blood donors, particularly premenopausal women. Providing iron supplements to donors with low iron stores or evidence of iron deficiency can help replenish iron reserves and prevent the decline in Hb levels. Adjusting donation intervals based on individual iron status and donation history can help minimize the risk of iron deficiency and anemia. Educating donors about the importance of iron for health and the potential impact of blood donation on Hb levels can empower them to make informed decisions about their donation frequency and iron intake.[15-17]

Erythrocyte indices, which include measurements of red blood cell size and hemoglobin content, provide valuable insights into the health and functional capacity of red blood cells. These indices are routinely assessed in blood donors to evaluate their hematological status and ensure the quality of blood products for transfusion. Our study revealed significant differences in MCV (Mean Corpuscular Volume) and MCH (Mean Corpuscular Hemoglobin) values among the different donation frequency groups, but not in MCHC (Mean Corpuscular Hemoglobin

Concentration) values. The decrease in MCV and MCH with increasing donation frequency suggests that frequent blood donation can lead to microcytic (smaller red blood cells) and hypochromic (red blood cells with reduced hemoglobin content) changes, which are characteristic of iron deficiency. Erythrocyte indices are a set of measurements that provide a quantitative description of red blood cell morphology, including size, hemoglobin content, and hemoglobin concentration. These indices are routinely measured as part of a complete blood count (CBC) and are used to assess various aspects of red blood cell health and function, aiding in the diagnosis and monitoring of various hematological conditions. MCV, or Mean Corpuscular Volume, measures the average volume of a red blood cell and is expressed in femtoliters (fL). MCV values can be classified as normocytic (normal size), microcytic (smaller than normal), or macrocytic (larger than normal). MCH, or Mean Corpuscular Hemoglobin, measures the average amount of hemoglobin per red blood cell and is expressed in picograms (pg). MCH values can be classified as normochromic (normal hemoglobin content), hypochromic (lower than normal hemoglobin content), or hyperchromic (higher than normal hemoglobin content). MCHC, or Mean Corpuscular Hemoglobin Concentration, measures the average concentration of hemoglobin in a given volume of packed red blood cells and is expressed as a percentage. MCHC values can be classified as normochromic (normal hemoglobin concentration), hypochromic (lower than normal hemoglobin concentration), or hyperchromic (higher than normal hemoglobin concentration). Erythrocyte indices are important parameters in blood donation for several reasons. First, they can help identify donors who may have underlying hematological conditions that could affect the quality of their blood donation. For example, donors with microcytic or hypochromic red blood cells may have iron deficiency anemia, which could compromise the oxygen-carrying capacity of their donated blood. Second, erythrocyte indices can help monitor the impact of blood donation on the donor's hematological health. Frequent blood donation can lead to a decrease in MCV and MCH, indicating the development of microcytic and hypochromic red blood cells, which can be an early sign of iron deficiency, even if the donor's hemoglobin level is still within the normal range. Third, erythrocyte indices can help distinguish between different types of anemia. For example, iron deficiency anemia is typically characterized by microcytic and hypochromic red blood cells, while thalassemia, a genetic disorder that affects hemoglobin production, is often associated with microcytic but normochromic red blood cells. Iron deficiency anemia is a common type of anemia that occurs when the body does not have enough iron to produce adequate amounts of hemoglobin. Hemoglobin is essential for carrying oxygen in the blood, and iron is a key component of hemoglobin. When the body lacks iron, it cannot produce enough hemoglobin, and the red blood cells become smaller and paler than normal. This is reflected in the erythrocyte indices as a decrease in MCV and MCH. The MCV is typically the first erythrocyte index to decrease in iron deficiency anemia. As iron deficiency worsens, the MCH also decreases. The MCHC usually remains normal in early iron deficiency anemia, but it may decrease in severe cases. Erythrocyte indices can also be helpful in distinguishing iron deficiency anemia from other types of anemia. For example, thalassemia, a genetic disorder that affects hemoglobin production, is often associated with microcytic but normochromic red blood cells. In thalassemia, the red blood cells are smaller than normal, but they contain a normal amount of hemoglobin. This is in contrast to iron deficiency anemia, where the red blood cells are both smaller and paler than normal. Other types of anemia, such as vitamin B12 deficiency anemia and folate deficiency anemia, are typically associated with macrocytic red blood cells. In these types of anemia, the red blood cells are larger than normal. Monitoring erythrocyte indices, in addition to hemoglobin levels, is important for assessing the hematological health of blood donors and ensuring the quality of blood products for transfusion. Frequent blood donors,

particularly premenopausal women, should be monitored for changes in erythrocyte indices that may indicate iron deficiency.[18-20]

5. Conclusion

This study investigated the impact of donation frequency on iron stores and hematological parameters in regular blood donors. The findings indicate that donation frequency significantly affects iron stores and hemoglobin levels in female blood donors, but not in male donors. Regular monitoring of iron stores, particularly in female donors, is crucial to prevent iron deficiency and anemia. Strategies to mitigate iron deficiency in frequent donors may include iron supplementation and adjustment of donation intervals based on individual iron status. Further research is needed to explore the long-term effects of frequent blood donation on iron stores and hematological health, and to develop evidence-based guidelines for donor monitoring and iron supplementation.

6. References

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