Original Article

Regular donor characteristics, inter-donation interval and the presence of subclinical anemia – A 3-year observational single-center study

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ABSTRACT

Regular blood donation is one of the potential causes of iron loss in donors. Understanding the primary causes of subclinical anemia will aid in promoting the well-being of blood donors and improve the steadily diminishing donor pool. This study aims to analyze the effect of regular donations and investigate the incidences of subclinical anemia and their essential risk factors. A retrospective analytic observational study was used, from January 2016-December 2019. Demographic information was collected. Males with a hemoglobin level between 13 and 13.9 g/dL and females with a hemoglobin level between 12 and 12.9 g/dL are considered to have subclinical anemia. The data were analyzed with comparative, bivariate, and regression tests using the SPSSv25 with a significance of p-value<0.05. The study included a total of 4,629 regular voluntary blood donors. The mean age was 31.2 ± 9.87 years (17–65), and the weight was 67.96 ± 12.40 kg. The subclinical anemia of male and female subjects was 25.9% vs. 35.4%. Donors with subclinical anemia were female (OR=1.567, p<.001), weight ≤ 63.5 kg (OR=1.527, p<0.001), inter-donation intervals 8-21 weeks (OR=1.447, p<0.001), previous donation hemoglobin levels ≤ 14.75 g/dL (OR=3.051, p<0.001), and several donations ≥ 6 times (OR=1.533, p<0.001). Risk of subclinical anemia was female, weight ≤ 63.5 kg, inter-donation interval 8-21 weeks, previous donation hemoglobin level ≤ 14.75 g/dL in males or ≤ 14 g/dL in females, and several donations ≥ 6 times.

Keywords: Regular donor, Demographic characteristics, Inter-donation interval, Hemoglobin levels, Subclinical anemia

Introduction

Blood transfusion is a central component of the medical and surgical care of patients [1-4] in all nations' healthcare systems. Its practice relies on the secure transfusion of blood from healthy donors to patients [5, 6]. Blood transfusions are only effective if the transfused blood is of high quality and the donor is secure.

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Regular blood donation is associated with donor iron deficiency [7]. Regular blood donation can contribute significantly to iron deficiency in blood donors. The movement of iron from body stores is caused by bleeding [8]. A person can either remain stable with low iron stores or become anemic during regular blood donations. Iron deficiency is commonly observed among long-term, consistent blood donors [9]. There has been a regulation of blood donation frequency to prevent anemia in the majority of donors, but quantitative data remains scarce [10, 11]. The screening of a donor's hemoglobin level is a standard component of the donor selection procedure to ensure that the red blood cells collected are of sufficient quality and that the donor is healthy [12].

Strategies to prevent hemoglobin deficiency and iron deficiency anemia, including donor reduction, have been implemented in many individual institutions [13]. In general, the minimum

interval between whole blood (WB) donations ranges from 56 days (8 weeks) to 16 weeks, and the World Health Organization recommends donating no more than once every two months [7, 14]. On the other hand, Increasing the interval between donations may adversely affect blood collection, depending on the extent of interval extension [15]. The interval between donations is one of the strongest predictors of iron deficiency [16]. Understanding the root causes of subclinical anemia to prevent delays and developing action plans to avoid it will help promote donor health and improve the donor shrinking pool. Recruiting and retaining regular blood donors requires diagnosing risk factors for subclinical anemia as a simple measure of iron status, and implementing essential iron supplementation procedures [17]. Considering all these reasons, this study was conducted. This study aimed to analyze the effects of regular blood donation and investigate the incidence of subclinical anemia and its essential risk factors [18].

Materials and Methods

This study used a retrospective design with a targeted sampling technique, from January 2016 to December 2019. The study was conducted at Dr. Sardjito Hospital's Blood Transfusion Center, Yogyakarta-Indonesia. Donor selection criteria followed national guidelines. The sample population consisted of all regular blood donors who had donated blood within the previous year and who continued to donate blood at least annually [19]. The number of donations was determined by calculating the cumulative number of donor identifiers in the data set, which represented each donor's donation during the period. In this study, the regular donors were further divided into four categories based on the donation number they made during the three-year study period. Group I, donors who donated three times; Group II, donors who have donated four to six times; Group III, donors who have donated seven to nine times; and Group IV, donors who have donated 10 or more times. Each donation's demographic information (sex, age, weight, blood group, and date of collection) was routinely collected and maintained in the database utilized for this study. The minimum inter-donation interval was ≥ 8 weeks (2 months) for whole blood donation. At each visit, donor hemoglobin is measured in fingertip samples using a hand-held hemoglobinometer (CompoLab, Fresenius Kabi, Bad Homburg, Germany). Males with a hemoglobin level between 13 and 13.9 g/dL and females with a hemoglobin level between 12 and 12.9 g/dL are considered to have subclinical anemia [20].

Analyses were conducted using the Statistical Package for the Social Sciences (SPSS) software, version 25.0 (IBM, Chicago, IL). Mean and standard deviation was used to convey descriptive statistics. The dataset's normality was evaluated using Kolmogorov-Smirnov analysis. The one-way variance analysis (ANOVA) was used to determine the statistical differences between numeric data, and using the Kruskal-Wallis test, categorical data were compared. Receiver operator characteristic (ROC) curves were used as acceptable cutoff values for assessing the usefulness of predictive markers of subclinical anemia. Using a Chi-Square test and Logistic Regression with interaction effects, the odds of asymptomatic anemia were examined. The level of significance was set to $p < 0.05$.

Results and Discussion

Between January 1, 2016, and December 31, 2019, the overall number of WB collections was 83,654 units from 49,191 donors. The study included a total of 4,629 regular voluntary blood donors. Based on the number of donations, group I consisted of 1,989 donors (43.0%), Group II consisted of 1,860 donors (40.2%), Group III consisted of 519 donors (11.2%), and Group IV consisted of 261 donors (5.6%) as subjects, respectively **(Table 1)**.

Table 1. The distribution of donors stratified by their characteristics and donation number

Characteristics	Donation number					p-value
	3	4-6	7-9	≥10	Total	
			Gender, n (%)			<0.001**
Male	1,450 (42.0)	1,355 (39.2)	424 (12.3)	226 (6.5)	3,455 (74.6)	
Female	539 (45.9)	505 (43.0)	95 (8.1)	35 (3.0)	1,174 (25.4)	
Age (Years)	31.21 ± 9.91	30.95 ± 9.95	31.41 ± 9.22	32.80 ± 10.03	31.22 ± 9.87	0.039*
Weight (kg)	68.09 ± 13.04	67.32 ± 11.71	68.88 ± 12.52	69.49 ± 11.64	67.96 ± 12.40	0.009*
			ABO Group, n (%)			0.019**
O	728 (43.5)	692 (41.3)	177 (10.6)	77 (4.6)	1,674 (36.2)	
A	486 (42.1)	469 (40.6)	130 (11.3)	69 (6.0)	1,154 (24.9)	
B	597 (42.7)	539 (38.6)	166 (11.9)	95 (6.8)	1,397 (30.2)	
AB	178 (44.1)	160 (39.6)	46 (11.4)	20 (5.0)	404 (8.7)	
			RhD group n (%)			0.753**
Positive	1,964 (42.9)	1,839 (40.2)	515 (11.3)	259 (5.7)	4,577 (98.9)	
Negative	25 (48.1)	21 (40.4)	4 (7.7)	2 (3.8)	52 (1.1)	

* One-way variance analysis (ANOVA); ** Kruskal-Wallis

The analysis of some demographic characteristics and donor types is presented in **Table 1**. Comparing the demographics of donors in different donation number categories, the proportion of male donors among those who made at least three donations was 74.6%. In contrast, the proportion of female donors was 25.4%. The difference between the distribution of male and female donors was statistically significant ($p < 0.05$). The subject's mean age was 31.2 ± 9.87 years with an interval of 17–65 years, and the mean weight was 67.96 ± 12.40 kg. The distribution of donation numbers based on donor types (ABO group) was found to differ statistically ($p < 0.05$).

Table 2 compares the inter-donation interval and hemoglobin levels of donors based on their gender and number of donations. Statistical disparities in inter-donation intervals (weeks) were observed between groups of donors. Since only regular donors who passed the hemoglobin screening were chosen, none of the subjects had clinical anemia. The subclinical anemia proportion of the male and female subjects was 25.9% and 35.4% respectively. The incidence of subclinical anemia based on donation number for each group I-IV in male subjects were 21.1%, 26.1%, 32.1%, and 44.7%, and in female subjects were 37.8%, 34.1%, 32.6%, and 25.7% respectively.

Table 2. Comparison of inter-donation intervals, hemoglobin level, and subclinical anemia stratified by gender and donation number

Variable	Donation number					p-value
	3	4–6	7–9	≥10	Total	
Male (n = 3,455)						
Inter-donation intervals (weeks)	33.22± 17.13	25.60± 10.17	17.68± 3.85	13.54± 2.12	27.04 ± 14.33	<0.001*
Hemoglobin levels (g/dL)						
Mean	15.19 ± 0.96	15.09 ± 0.90	14.98 ± 0.84	14.80 ± 0.82	15.10 ± 0.92	<0.001*
First time	15.43 ± 1.15	15.49 ± 1.13	15.67 ± 1.06	15.71 ± 1.07	15.50 ± 1.13	<0.001*
Last time	14.97 ± 1.21	14.77 ± 1.21	14.58 ± 1.25	14.26 ± 1.25	14.80 ± 1.23	<0.001*
Subclinical anemia, n (%)						<0.001**
Yes	306 (21.1)	353 (26.1)	136 (32.1)	101 (44.7)	896 (25.9)	
No	1,144 (78.9)	1,002 (73.9)	288 (67.9)	125 (55.3)	2,559 (74.1)	
Female (n = 1,174)						
Inter-donation intervals (weeks)	31.35 ± 16.03	25.34 ± 9.92	18.05 ± 3.47	14.40 ± 2.39	27.18 ± 13.51	<0.001*
Hemoglobin levels (g/dL)						
Mean	13.56 ± 0.64	13.58 ± 0.62	13.59 ± 0.54	13.83 ± 0.90	13.58 ± 0.64	0.115*
First time	13.82 ± 0.89	13.97 ± 0.96	14.20 ± 1.00	14.41 ± 1.28	13.93 ± 0.95	<0.001*
Last time	13.36 ± 0.81	13.40 ± 0.83	13.29 ± 0.78	13.63 ± 1.03	13.38 ± 0.82	0.179*
Subclinical anemia, n (%)						0.203*
Yes	204 (37.8)	172 (34.1)	31 (32.6)	9 (25.7)	416 (35.4)	
No	335 (62.2)	333 (65.9)	64 (67.4)	26 (74.3)	758 (64.6)	

* One-way variance analysis (ANOVA); ** Kruskal-Wallis

Hemoglobin levels both in males and females were decreased when the donation number increased **(Figure 1)**. There is also a shifting pattern of distribution of hemoglobin levels among male and female donors in distinct donation number categories. Regular male donors exhibited a distinct fluctuating pattern, whereas regular female donors exhibited no distinct pattern.

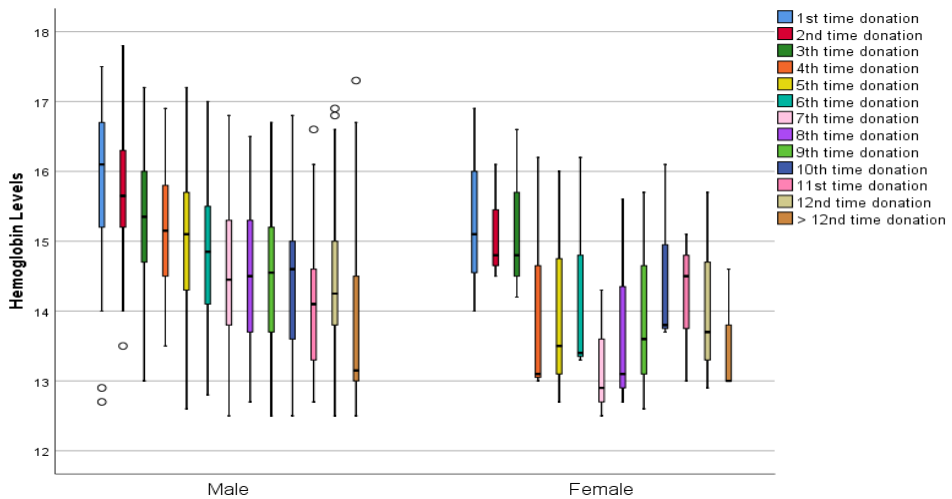

Figure 1. The hemoglobin distribution of male and female regular donors stratified by donation number

Age, weight, inter-donation interval, and previous donation hemoglobin levels converted by the receiver operator characteristic (ROC) curve for acceptable cutoff were used to determine the intersection point of subclinical anemia. **Table 3** displays the results of univariate and multiple logistic regression analysis. Risk factors identified as predicting subclinical anemia were female (OR=1.567, p<0.001), weight ≤ 63.5 kg (OR=1.527, p<0.001), the inter-donation intervals 8-21 weeks (OR=1.447, p<0.001), the previous donation hemoglobin level ≤ 14.75 g/dL (OR=3.051, p<0.001), and the number of donations ≥ 6 times (OR=1.533, p<0.001). Previous donation hemoglobin levels were the highest risk factor for present subclinical anemia.

We further elucidated the cause of differences in subclinical anemia between males and females **(Table 3)**. Male regular donors with weight ≤ 63.5 kg, inter-donation intervals 8-21 weeks, previous blood donation hemoglobin levels ≤ 14.75 g/dL and the number of donations ≥ 6 times were associated with an increased risk of subclinical anemia. Female regular donors with weight ≤ 63.5 kg, inter-donation intervals 8-30 weeks, and previous blood donation hemoglobin levels ≤ 14 g/dL were associated with an increased risk of subclinical anemia.

Table 3. Univariate and multivariate analysis of the risk of subclinical anemia in regular donors and stratified by gender

Variable	Univariate			Multivariate		
	OR	95% CI	p-value	OR	95% CI	p-value
Female	1.567	1.360-1.806	<0.001	1.316	1.109-1.563	0.002
Age ≥ 37.5 years	1.035	0.994-1.078	0.109	0.980	0.835-1.150	0.805
Weight ≤ 63.5 kg	1.527	1.340-1.746	<0.001	1.347	1.171-1.549	<0.001
IDI 8-21 week	1.447	1.273-1.645	<0.001	1.259	1.091-1.452	0.002
Previous donation hemoglobin level ≤ 14.75 g/dL	3.051	2.674-3.482	<0.001	3.497	2.994-4.084	<0.001
Number of donations≥ 6 times	1.533	1.328-1.769	<0.001	1.656	1.409-1.947	<0.001
Male						
Weight ≤ 63.5 kg	1.360	1.160-1.594	<0.001	1.259	1.064-1.489	0.007
IDI 8-21 weeks	1.560	1.338-1.817	<0.001	1.221	1.027-1.453	0.024
Previous donation hemoglobin level ≤ 14.75 g/dL	3.692	3.126-4.361	<0.001	3.917	3.299-4.651	<0.001
Number of donations ≥ 6 times	1.860	1.577-2.194	<0.001	1.998	1.653-2.414	<0.001
Female						
Weight ≤ 63.5 kg	1.544	1.195-1.996	<0.001	1.503	1.160-1.948	0.002
IDI 8-30 weeks	1.381	1.065-1.791	0.018	1.419	1.090-1.846	0.009
Previous blood donation hemoglobin level ≤ 14 g/dL	1.694	1.318-2.176	<0.001	1.688	1.311-2.173	<0.001
Number of donations ≥ 6 times	1.013	0.913-1.125	0.864	-	-	-

IDI: Inter-donation intervals

Our study aims to identify the characteristics of regular blood donors by analyzing their demographics and hemoglobin levels stratified by their number and sex. The age distribution ranged from 17 to 65, with a mean of 31,2 9,87 years, indicating that younger donors also contributed to helping the donation of blood. This finding reflected the demographic structure of Indonesian donors. Male donors were predominantly involved, whereas female donors were discouraged from donating blood due to their monthly menstrual cycles [3].

According to **Table 1**, O+ve donors were more likely to be consistent donors. This can be attributed to the fact that O+ve blood can be used in patients with other blood groups, and as a result, these donors may be more willing to give. The literature has not yet established a connection between blood type and the number of donors. One study found no significant gender, blood type, Rh blood group, or blood collection unit differences in the proportion of non-returned donors [21]. A multivariate analysis of the yield rate of first-time donors revealed that younger (17-year-old) and elderly (50+-year-old) donors, males, blood group O donors, and those with no initial adverse reaction were most likely to return [22].

Triple donors had substantially greater hemoglobin levels than those who had donated three or more times, even though the latter were within the acceptable range. This result aligns with previous studies [23, 24]. In this study, the consistent pattern of decreasing hemoglobin levels as the donation number increases were shown in males but not in females. According to multiple cross-sectional studies of blood donors, including the RISE study, more frequent donations result in greater iron loss and are the most significant factor leading to iron deficiency [25]. The time required to restore the iron lost during donation depends on several variables, including the amount of blood donated, the donor's iron status at the time of donation, his or her age, and sex. Long-term observations are required to determine the effect of this alteration on the overall hemoglobin levels of male donors. Many factors lead to low hemoglobin levels, especially in women. In addition to blood donation, there were significant correlations between protein intake, iron content, menstrual cycle, and length of menstruation with hemoglobin levels [26].

All donors were assessed as non-anemic based on hemoglobin levels during donation. However, to protect regular donors from iron deficiency, the results of this study raised concerns regarding the use of biomarkers beyond hemoglobin level before donation,

particularly among those who have donated more than three times. Previous hemoglobin levels ≤ 14.75 g/dL in males and ≤ 14 g/dL in females had an increased risk of subclinical anemia in regular donors.

A blood center's primary responsibility is to safeguard both the donor and the patient. A unit of blood (450mL) contains nearly 250 mg of iron, which accounts for approximately 30% of the average body iron stores (BIS) in males and nearly 80% in females [8]. In the early stages of iron deficiency, the estimation of hemoglobin level is less accurate [27]. Other biochemical markers, including serum ferritin, soluble transferrin receptors, serum iron, and TIBC, would have aided in a more accurate interpretation of iron status.

Inter-donation intervals between 8-21 weeks and the number of donations ≥ 6 times had an increased risk of subclinical anemia. Regular donation increases the risk of iron [28] deficiency, which would harm hemoglobin. The essential eligibility criteria for the number of donations, the minimum interval between donations, and the hemoglobin cutoff levels vary between jurisdictions [29]. Observational studies on iron deficiency risk factors conducted in several nations, including Canada, the United States, Denmark, Australia, and the Netherlands, indicate that a high frequency of donation is a significant risk factor for iron deficiency [30]. Iron supplementation is an alternative to increasing the frequency of mutual donations and subsequently losing the most devoted blood donors. The study by Newman *et al*. [31] provided compelling evidence for short-term iron supplementation. Iron supplementation in blood donors has been extensively discussed in the United States and abroad. However, there are no standard practices in place [31]. Furthermore, this may not be sufficient to prevent iron deficiency in women of reproductive age who donate blood every eight weeks.

The limitation of this study was donors who had deficient stores. However, on further donations, the average hemoglobin level or subclinical anemia would eventually develop into iron deficiency anemia.

Conclusion

Regular donors with a higher frequency of donations have substantially lower hemoglobin levels. Risk of subclinical anemia was in females, weight ≤ 63.5 kg, inter-donation intervals 8-21 weeks, previous hemoglobin level ≤ 14.75 g/dL in males or ≤ 14 g/dL in females, and donation number ≥ 6 times. Consideration should be given to the measurement of iron status and the necessary measures for iron supplementation, focusing on regular donors at risk of subclinical anemia.

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Conflict of interest: None

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