RESULTS OF IRON SUPPLEMENTATION FOR REGULAR VOLUNTARY NON-REMUNERATED BLOOD DONORS AT NATIONAL INSTITUTE OF HEMATOLOGY AND BLOOD TRANSFUSION

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SUMMARY

Objective: *To evaluate the results of iron supplementation for RRVBD at the National Institute of Hematology and Blood Transfusion.* ***Subjects and methods:*** *Including 279 RRVBD with serum ferritin below 26 ng/ml taking iron tablets containing 35 mg of elemental iron. Cross-sectional description, prospective study.* ***Results****: The proportion of RRVBD taking iron tablets and testing on time is 43.4%, the proportion of RRVBD taking enough iron tablets and not coming to the test on time is 18.6%. Hematological parameters of RRVBD taking iron tablets are within the normal range. The group of RRVBD taking enough iron tablets and testing on time: the mean serum ferritin concentration before taking iron tablets is 15.8 ± 5.5 ng/ml and after taking iron tablets is 24.7 ± 14.9 ng/ml, the proportion of RRVBD with ferritin returning to normal range after taking iron tablets is 39.7%. In group of RRVBD not taking iron tablets or not taking enough iron tablets, serum ferritin before iron intake is 18.1 ± 5.3 ng/ml and after iron intake is 14.2 ± 10.9 ng/ml, the proportion of blood donors having serum ferritin returning to normal range after iron intake is 7.8%. In group of RRVBD taking enough iron tablets and not coming to the test on time, the mean serum ferritin concentration before iron intake is 16.5 ± 5.9 ng/ml and after iron intake is 28.8 ± 17.1 ng/ml, the proportion of RRVBD with serum ferritin concentration returning to normal range after blood donation is 51.9%.* ***Conclusion****:The RRVBD group, upon consuming sufficient iron and undergoing timely examinations, displayed an increased average serum iron concentration compared to their levels prior to iron supplementation, whereas the subgroup that either lacked adequate iron intake or took insufficient iron exhibited a decreased average serum ferritin concentration compared to their levels prior to initiating iron intake.*

Keywords*: regular repeated blood donors, serum ferritin.*

1. INTRODUCTION

Blood and blood products are invaluable medicines, essential for emergency and specialized treatment of diseases that require blood transfusion. Diseases such as blood clotting disorders, congenital bleeding disorders, blood cancers, and others necessitate blood transfusions. Blood can only be obtained from voluntary blood donors, as there is currently no substitute source. Therefore, caring for and maintaining a steady supply of blood donors is of utmost importance, especially focusing on iron supplementation for regular repeated blood donors (RRVBD) [1], [2].

Each milliliter of blood contains about 0.5 mg of iron. Consequently, with each blood donation, a certain amount of iron is lost by the blood donor. Many countries

around the world are concerned about providing iron supplementation for RRVBD. However, as of now, this practice has not been implemented in our country [1], [2].

Therefore, the research topic is pursued with the following objective: "Assessment of iron supplementation outcomes for regular repeated blood donors at the National Institute of Hematology and Blood Transfusion."

2. 2. SUBJECTS AND METHODS

2.1. Patient cohort

Including 279 RRVBD with serum ferritin below 26 ng/ml taking iron tablets containing 35 mg of elemental iron.

2.2. METHODS

***2.2.1. Research design**:* Cross-sectional description, prospective study.

2.2.2. Method of procedure

- Selection criteria: The eligible blood donors are those who meet the blood donation eligibility criteria outlined in Circular 26/BYT/2013 [3]. The potential blood donors' comprehensive blood cell analysis, serum iron concentration, and serum ferritin levels are examined. Donors with serum ferritin levels < 26 ng/ml are chosen for advising on taking iron supplements after blood donation.
- RRVBD for male donors is a minimum of 3 times per year, and for female donors, it's a minimum of 2 times per year.
- According to Joseph E. Kiss in 2015, blood donors with ferritin levels < 26 ng/ml are considered as having decreased ferritin (iron deficiency), while those with ferritin levels ≥ 26 ng/ml are considered normal (not iron deficient) [4].
- Sample size for blood donors receiving iron supplements: Based on the formula estimating a proportion: $n = Z^2_{1-\alpha/2} \times p(1-p) \times d^2$
  - n: is the sample size
  - p: is the proportion of individuals with decreased ferritin (according to the AABB report in 2017, the proportion of decreased ferritin is 0.22).
  - d: is the desired margin of error between the proportion obtained from the study sample and the population proportion, with d = 0.05 chosen.
  - α: is the significance level, Z 1- α/2 is the confidence coefficient, and the value of Z obtained from the Z-table corresponding to the chosen α value, α = 0.05 (95% confidence interval), the Z value being 1.96.
  - Using the formula, the calculated sample size for blood donors using iron supplements is n ≥ 263.

2.2.3. Research content

- RRVBD with serum ferritin below 26 ng/ml are advised to take iron supplementation:
  - Use a type of iron tablet: Take a tablet containing 300 mg of ferrous gluconate, equivalent to 35 mg of elemental iron, take 1 tablet on an empty stomach within 60 days.
  - RRVBD is invited to the Institute of Hematology and Blood Transfusion for a follow-up test after 85 days from the start of medication, and the maximum sampling time is 120 days.
- The follow-up tests after taking iron tablets include: Complete blood cell analysis, serum iron concentration, and serum ferritin.
- Classification of RRVBD subjects:
  - Group 1: Blood donors who took iron tablets and came for a follow-up test including complete blood cell analysis, serum iron, and serum ferritin within the timeframe from day 85 to under 120 days from the donation date.
  - Group 2: Blood donors who did not take iron or took insufficient iron supplementation, and came for a follow-up test including complete

blood cell analysis, serum iron, and serum ferritin, with the testing time starting from day 85 after the donation date.

+ Group 3: Blood donors who took sufficient iron tablets and came for a follow-up test including complete blood cell analysis, serum iron, and serum ferritin after 120 days from the donation date.
+ Group 4: Blood donors who withdraw from the study, do not take iron tablets, and do not repeat the tests.

– Evaluation of RRVBD's test results after taking supplemental iron.

2.3. Data processing: SPSS 20.0

3. RESULTS

Table 1. Some features of RRVBD using iron tablets at NIHBT

The individuals who donate blood take iron tablets	Male		Female		Total	
	n	%	n	%	n	%
Group 1 (RRVBD taking iron tablets and testing on time)	25	***9.0***	96	***34.4***	121	***43.4***
Group 2 (RRVBD not taking iron tablets or not taking enough iron tablets)	4	***1.4***	47	***16.8***	51	***18.3***
Group 3 (RRVBD taking enough iron tablets and not coming to the test on time)	5	***1.8***	47	***16.8***	52	***18.6***
Group 4 (RRVBD dropping out of the study)	2	***0.7***	53	***19.0***	55	***19.7***
Total	36	***12.9***	243	***87.1***	279	***100***

There were 279 regular repeated blood donors who agreed to participate in an iron supplementation program, among whom 121 RRVBD took the iron supplements fully and had their tests done on time, resulting in a rate of 43.4%. RRVBD who did not take the iron supplements or took them incompletely and underwent retesting were 51 individuals, accounting for a rate of 18.3%. RRVBD who took the iron supplements fully but had their tests done late were 52 individuals, making up a rate of 18.6%. RRVBD who received the iron supplements but neither took them nor underwent the test were 55 individuals, representing a rate of 19.7%.

Table 2. Characteristics regarding age, number of blood donations, and post-iron supplementation testing time of the RRVBD subject taking iron tablets

RRVBD subject taking iron tablets	Age	Number of blood donations	Post-iron supplementation testing time
	Av ± SD	Av ± SD	Av ± SD
Group 1 (n = 121)	28.9 ± 7.7	12.6 ± 8.9	96.6 ± 9.7
Group 2 (n = 51)	25.8 ± 7.4	11.7 ± 8.6	322.2 ± 274.9
Group 3 (n = 52)	27.2 ± 7.1	9.8 ± 8.6	189.5 ± 115.3
Group 4 (n = 55)	27.6 ± 7.1	7.6 ± 4.2	0
Total (n= 279)	27.8 ± 7.5	11.0 ± 7.7	169.5 ± 168.4
p	p1-2 < 0.05 p1-3, p2-3 ≥ 0.05	p1-2, p2-3 ≥ 0.05 p1-3, p1-4< 0.05	p1-2, p1-3, p2-3 < 0.05

RRVBD subjects taking iron tablets have an average age of 27.8 ± 7.5 years, an average number of blood donations of 11.0 ± 7.7 times, and an average post-iron

supplementation testing time of 169.5 ± 168.4 days. The RRVBD group that fully complied with iron intake and participated in testing at the correct average time was 96.6 ± 9.7 days. In contrast, the RRVBD group that did not fully comply with iron intake or did not take iron had the longest average testing time of 322.2 ± 274.9 days, with statistically significant differences at $p < 0.05$.

Table 3. Certain hematological parameters, serum iron levels, and ferritin levels of RRVBD prior to iron tablet supplementation

Parameters (n=279)	Group 1	Group 2	Group 3	Group 4	p
	Av ± SD (n=121)	Av ± SD (n=51)	Av ± SD (n=52)	Av ± SD (n=55)	
RBC (T/l)	4.63 ± 0.44	4.61 ± 0.38	4.56±0.44	4.57±0.58	≥ 0.05
HBG (g/l)	130.4 ± 11.2	128.9 ± 6.9	128.1 ± 8.7	128.6±8.7	≥ 0.05
MCV (fl)	86.4 ± 5.6	86.4 ± 5.6	85.9 ± 6.0	87.7±4.9	≥ 0.05
MCH (pg)	28.2 ± 2.2	28.1 ± 2.1	28.1 ± 2.3	28.3±2.8	≥ 0.05
Fe (µmol/L)	12.8 ± 6.1	15.1 ± 8.2	14.9 ± 6.6	13.2 ±7.2	≥ 0.05
Ferritin (ng/ml)	15.8 ± 5.5	18.1 ± 5.3	16.5 ± 5.9	15.4 ± 5.4	≥ 0.05

Some hematological indices, average serum iron of RRVBD, lie within the range of normal individuals, while the average ferritin concentration of RRVBD is 15.4 ± 5.4 ng/ml, lower than the normal range (selected standard below 26 ng/ml). Hematological indices, serum iron concentration, and average serum ferritin levels showed no significant differences among RRVBD groups before iron tablet supplementation.

Table 4. The results of examining certain hematological indices, serum iron concentration, and ferritin levels of RRVBD after iron supplementation

Parameters (n=279)	Group 1	Group 2	Group 3	p
	Av ± SD (n=121)	Av ± SD (n=51)	Av ± SD (n=52)	
RBC (T/l)	4.75 ±0.51	4.58 ± 0.39	4.67 ± 0.46	≥ 0.05
HBG (g/l)	132.6 ± 11.6	126.2 ± 6.7	132.4 ± 9.9	< 0.05
MCV (fl)	85.3 ± 5.4	85.0 ± 5.5	85.7 ± 5.7	≥ 0.05
MCH (pg)	27.9 ± 2.0	27.6 ± 2.1	28.3 ± 2.2	≥ 0.05
Fe (µmol/L)	16.0 ± 9.3	13.9 ± 10.5	15.4 ± 7.1	≥ 0.05
Ferritin (ng/ml)	24.7 ± 14.9	14.2 ± 10.9	28.8 ± 17.1	< 0.05

The quantities of red blood cells, HBG, MCV, MCH, and serum iron concentration in the RRVBD groups of blood donors after iron tablet supplementation all fall within the normal range. The average serum ferritin concentration in the RRVBD group that used complete iron tablets (group 1) and adhered to scheduled testing, as well as the RRVBD group that did not take iron or took incomplete doses (group 2), is lower than that of normal individuals. The average hemoglobin concentration and average serum ferritin level of RRVBD subjects who did not use iron or used incomplete iron supplementation are lower than those of other RRVBD groups, with statistically significant differences at $p < 0.05$.

Table 5. Recovery outcomes of ferritin in RRVBD subjects after iron supplementation

	Normal and elevated ferritin levels	Decreased ferritin levels < 26 ng/ml	Total

	≥ 26 ng/ml					
	n	%	n	%	n	%
Group 1 (RRVBD taking iron tablets and testing on time)	48	***39.7***	73	***60.3***	121	100
Group 2 (RRVBD not taking iron tablets or not taking enough iron tablets)	4	***7.8***	47	***92.2***	51	100
Group 3 (RRVBD taking enough iron tablets and not coming to the test on time)	27	***51.9***	25	***48.1***	52	100
p	***< 0.05***					

The proportion of RRVBD subjects in the group of full iron intake and timely testing with normal and elevated ferritin levels is 39.7%, while for RRVBD subjects who do not take iron or take it irregularly, it is 7.8%. The group of RRVBD subjects taking full iron supplementation but not adhering to scheduled testing constitutes 51.9%. There is a statistically significant difference among the RRVBD groups with regards to iron tablet intake, with $p < 0.05$.

4. DICUSSION

Table 1 results show that out of the 279 RRVBD subjects with serum ferritin levels below 26 ng/ml, 121 RRVBD subjects adhered to both full iron tablet intake and timely testing, constituting a rate of 43.4%. Among the RRVBD subjects who did not take iron or took it inadequately and still underwent testing (51 individuals), the rate was 18.3%. For RRVBD subjects who took full iron supplementation but did not adhere to scheduled testing (52 individuals), the rate was 18.6%. Meanwhile, among the RRVBD subjects who neither took iron nor repeated the testing (55 individuals), the rate was 19.7%. Consequently, despite being advised and provided with free iron tablets for blood donation, a number of individuals did not provide information or failed to take the iron tablets adequately. According to the study by Walter Bialkowski et al. conducted in 2017, where 139 blood donors were supplemented with 38 mg of iron element within 60 days, there were 3 individuals who did not participate in the study and 17 blood donors dropped out, resulting in a dropout rate of 14.4%. In the case of supplementing 19 mg of iron element to 139 blood donors within 60 days, 6 individuals dropped out and 22 blood donors dropped out mid-study, with a dropout rate of 20.1% [5]. Therefore, the implementation of iron supplementation for RRVBD encounters a certain proportion of blood donors who either do not participate in the study or drop out mid-study, with various reasons including busy work schedules, medication forgetfulness, and occurrences of complications such as constipation, skin rashes, and others.

Table 2 results indicate that the mean age of RRVBD participants engaged in iron supplementation is 27.8 ± 7.5 years. The overall mean number of blood donations for RRVBD is 11.0 ± 7.7 times. Among these, blood donors who take complete iron supplementation and adhere to scheduled testing have an average of 12.6 ± 8.9 blood donation occurrences, which is higher than the group of blood donors who do not take iron or take it inadequately, with an average of 9.8 ± 8.6 blood donation occurrences. The group of blood donors who neither take iron nor undergo testing has an average of 7.6 ± 4.2 blood donation occurrences, and there is a statistically significant difference with $p < 0.05$. Therefore, RRVBD

individuals with more frequent blood donations are more likely to show an interest in iron supplementation compared to those with fewer blood donation occurrences.

Table 3 results demonstrate that the hematological indices including RBC, HBG, MCV, MCH, and average serum iron concentration all fall within the range of normal individuals, with no significant differences observed among the RRVBD subject groups taking iron tablets ($p \geq 0.05$). The average serum ferritin concentration among the RRVBD subject groups taking iron tablets is below 26 ng/ml, and there are no significant differences among the groups ($p \geq 0.05$). Therefore, several hematological indices and average serum iron and ferritin concentrations before blood donation in the various RRVBD subject groups prior to iron tablet usage exhibit no differences.

Table 4 results reveal that several hematological indices and serum iron concentrations of RRVBD after iron supplementation fall within the range of normal individuals. However, in the group of RRVBD subjects who did not use iron or used it inadequately and underwent testing after iron supplementation, the HBG was 126.2 ± 6.7 g/l, which is lower than the group of RRVBD subjects using complete iron supplementation and adhering to scheduled testing, with an HBG of 132.6 ± 11.6 after iron supplementation, and the group of RRVBD subjects using complete iron supplementation but not adhering to scheduled testing, with an HBG of 132.4 ± 9.9 g/l. There is a statistically significant difference among the RRVBD subject groups taking iron tablets with $p < 0.05$. Regarding the average serum ferritin concentration in the group of RRVBD subjects using complete iron supplementation but not adhering to scheduled testing, the average ferritin concentration after testing following iron supplementation is 28.8 ± 17.1 ng/ml, which is within the normal range for serum ferritin levels in normal individuals. This is in contrast to the average ferritin level before iron supplementation, which was 16.5 ± 5.9 ng/ml. In the group of RRVBD subjects using complete iron supplementation and adhering to scheduled testing, the average ferritin concentration after iron supplementation testing is 24.7 ± 14.9 ng/ml, which is not within the normal range (≥ 26 ng/ml); however, there is a significant improvement compared to the average ferritin level before iron supplementation, which was 15.8 ± 5.5 ng/ml. For the group of RRVBD subjects not taking iron tablets or taking them inadequately, the average serum ferritin concentration is 14.2 ± 10.9 ng/ml, which is even lower than the average ferritin level before iron supplementation, which was 18.1 ± 5.3 ng/ml. Therefore, there is a significant recovery in average serum ferritin concentration for RRVBD subjects using complete iron supplementation, while RRVBD subjects not taking iron or taking it inadequately do not exhibit improvement. According to a study by author Walter Bialkowski and colleagues in 2017, there was an improvement in total body iron content for RRVBD individuals in the group using 19 mg and 38 mg of iron element supplementation daily for 60 days. The author also suggested that using lower iron doses for blood donors compared to iron-deficient anemia treatment for patients (with a dose of 65 mg of iron element daily) reduces gastrointestinal side effects, leading to better adherence to iron supplementation [5]. According to author Moretti Diego and colleagues in 2015, continuous daily iron tablet use may not be as effective in absorption, and the author recommended that iron tablets can be taken every other day or even weekly [6].

Table 5 results show that the proportion of RRVBD individuals with ferritin concentrations ≥ 26 ng/ml in the group of blood donors who took complete iron supplementation and adhered to scheduled

testing is 39.7%. In the group of blood donors who did not use iron or used it inadequately, this proportion is 7.8%, and in the group of blood donors who took complete iron supplementation but did not adhere to scheduled testing, the proportion is 51.9%. The proportion of RRVBD individuals with ferritin concentrations ≥ 26 ng/ml is higher in the first group compared to the other two groups, with a statistically significant difference of $p < 0.05$. For RRVBD subjects who used complete iron supplementation but did not adhere to scheduled testing, the average time to retest after supplementation was 189.5 ± 115.3 days, whereas the group of RRVBD subjects who took complete iron supplementation and adhered to scheduled testing had an average time to retest of 96.6 ± 9.7 days, and the group of RRVBD subjects who did not use iron or used it inadequately had an average time to retest of 322.2 ± 274.9 days. Therefore, RRVBD individuals who used complete iron supplementation and had a longer interval to retest exhibited a higher proportion of serum ferritin recovery compared to the other two groups. According to a study by author Alan E. Mast and colleagues in 2020, the effect of taking iron tablets with 37.5 mg of iron element helped recover iron stores and iron in red blood cells at a minimum in blood donors with serum ferritin levels ≥ 50 ng/ml, but significant recovery occurred when serum ferritin was < 50 ng/ml. The study found that initial erythropoietin levels increased rapidly, nearly doubling after whole blood donation. After reaching the initial peak, erythropoietin levels decreased to baseline levels. This decrease was slowed down in the group of blood donors with serum ferritin levels below 12 ng/ml and between 12-50 ng/ml in those not taking iron compared to the iron-taking groups. As erythropoietin responds to blood oxygen deficiency, the slower decrease in those who donated blood may reflect lower HBG levels and a relatively prolonged cellular oxygen deficiency in the kidneys. The initial increase in erythropoietin was related to a corresponding increase in reticulocyte count. Reticulocytes remained elevated for a longer duration in the iron-taking groups, indicating an immediate reticulocyte response dependent on available iron and erythropoietin. Reticulocyte count then began to rise again around day 100 in individuals with serum ferritin levels ≥ 12 ng/mL. It's unclear why a similar response wasn't observed in individuals with ferritin levels < 12 ng/mL, but it could be related to continued low iron reserves in these blood donors. The authors indicated that blood donors will fully recover iron after 100 days of iron supplementation [7].

5. CONCLUSION

RRVBD subjects who took complete iron supplementation and adhered to scheduled testing constituted 43.4% of the group, while those who did not take iron or took it inadequately accounted for 18.3%. RRVBD individuals who took complete iron supplementation but did not adhere to scheduled testing made up 18.6%, and those who did not take iron and did not undergo testing comprised 19.7%.

Hematological indices including RBC, HBG, MCV, MCH, and serum iron concentrations of various RRVBD groups before and after iron supplementation all fell within the normal range.

Results of ferritin recovery in RRVBD after iron supplementation: Among RRVBD individuals who took complete iron supplementation and adhered to scheduled testing, the average serum iron concentration after supplementation was 24.7 ± 14.9 ng/ml, showing an increase compared to before iron intake. The proportion of blood donors with ferritin levels above 26 ng/ml was 39.7%. In the group of RRVBD individuals who did not take iron or took it inadequately, the average ferritin concentration was 14.2 ± 10.9 ng/ml, which was lower compared to

before iron intake, and the proportion of blood donors with ferritin levels above 26 ng/ml was 7.8%. For RRVBD individuals who took complete iron supplementation but did not adhere to scheduled testing, the average ferritin concentration was 28.8 ± 17.1 ng/ml, within the range of normal values, and the proportion of blood donors with ferritin levels above 26 ng/ml was 51.9%.

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