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Analysis of Reticulocyte Hemoglobin Equivalent in Routine and Non-Routine Blood Donors in Makassar

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ABSTRACT

Blood donation often causes the depletion of iron stores in the body resulting in anemia. One of the markers to assess the hemoglobin content in reticulocytes is Ret-He. This study aims to analyze Ret-He in routine and non-routine blood donors. This was a cross-sectional study using primary data from routine and non-routine blood donors at the Blood Transfusion Technical Implementation Unit of South Sulawesi Province from June 2021 to September 2021. The study population was blood donors who met the donor selection requirements. The research samples were blood donors who qualified as routine and non-routine blood donors. For a total of 66 blood donors, namely 33 routine blood donors and 33 non-routine blood donors, the mean Ret-He of routine blood donors (33.6 pg) was lower than that of non-routine blood donors (35 pg). The Mann-Whitney test showed a significant difference between routine and non-routine blood donors (p=0.008). Ret-He levels in routine blood donors were significantly lower than in non-routine blood donors. This is because routine blood donors donate blood more frequently and regularly, affecting the iron reserves in the donor's body. The study showed that the Ret-He level of routine blood donors was lower than that of non-routine blood donors but was still within the normal values of Ret-He (normal value: 30.2 pg - 36.7 pg). Ret-He levels in routine blood donors were lower than non-routine blood donors but were still within the normal range.

Keywords: Ret-He equivalent, routine blood donor, non-routine blood donor

INTRODUCTION

A blood donor is a person that donates their blood or blood components to patients with the purpose to treat a disease and health recovery.[1,2] Blood donors are divided into two, routine blood donors and non-routine blood donors. According to the Regulation of the Minister of Health number 91, the year 2015, the donation interval of a donor is every eight weeks with a frequency of 6 times a year for males and 4 times a year for females, and a donor will be deemed routine if they donate their blood at least two times a year.[2]

There are more than 9 million volunteer donors each year in the United States of America and almost 70% are routine donors, many regular donors have iron deficiency. Females are three times more likely to have iron deficiency than males. Thirty-five percent of the donor population in the USA is estimated to have iron deficiency.[3]

Blood donation can cause a 200–250 mg loss of iron that cause a depletion in iron reserves, which can lead to anemia. One of the indicators of assessing the content and storage of iron in the body is ferritin, but it is time-consuming and expensive.[4]

Reticulocytes are immature erythrocytes that stay 1–2 days in the peripheral blood and provide a good index of Hemoglobin (Hb) in producing new erythrocytes and responding to iron supplementation. Several markers to evaluate the Hb concentration in reticulocytes are reticulocyte hemoglobin equivalent (Ret-He) and reticulocyte hemoglobin content (CHr). Reticulocyte hemoglobin equivalent can be measured with the latest automatic hematology analyzer and is said to reflect the iron content in reticulocytes and is a direct index of cellular iron availability. Reticulocyte hemoglobin content and Ret-He correlates with iron deficiency and is a useful marker for iron deficiency in babies, kids, adult blood donors, geriatric patients, pregnant female, and patients with chronic kidney disease undergoing hemodialysis. According to a study by Toki *et al.*, Ret-He can be a clinically useful marker to determine an iron deficiency in the general population. The measurement of Ret-He is more beneficial than other parameters such as serum

ferritin for patients with anemia because the measurement is fast, automatic and only needs an EDTA blood sample.[5]

Ret-He can be used as a routine test in blood donors that gives a chance for appropriate intervention such as diet adjustment or iron supplements in donors.[6] According to Chinudomwong *et al.* the cut-off of Ret-He is 30 pg and has 96% sensitivity, 97.4% specificity 97.4%, 80% PPV, and 99.6% NPV in diagnosing iron deficiency anemia. Ret-He > 30 pg is used to rule out iron deficiency anemia due to good diagnostic sensitivity and specificity.[7] Therefore Ret-He examination should be done routinely on routine and non-routine blood donors to monitor iron deficiency in blood donors.

Research on Ret-He in blood donors has never been done in South Sulawesi and has never been conducted in an Indonesian population, especially Makassar. This study aims to analyze the difference in Ret-He concentration in routine blood donors and non-routine blood donors.

METHODS

This was a cross-sectional study using primary data from routine and non-routine donors at Blood Transfusion Technical Implementation Unit (Unit Pelaksana Teknis/UPT) in South Sulawesi Province from June 2021 to September 2021. The study population was blood donors who met the donor selection requirements at the South Sulawesi Province Blood Transfusion UPT. The research samples were blood donors who had met the requirements as routine and non-routine blood donors according to the attachment of the Minister of Health of the Republic of Indonesia Regulation number 91 of 2015. Donors who consumed all forms of iron supplements before donating their blood were excluded.

Routine donors, namely donors who donate blood regularly with a frequency of at least 4 times per year in the last 2 years, and non-routine donors, namely blood donors who donated blood in the previous year or before.

Ret-He levels examinations were carried out by taking the venous blood of the donor and measured with the flow cytometer method using the Sysmex XN-350 Hematology Analyzer indicated in picogram units (pg). The data were then analyzed statistically using SPSS version 25. The data normality test used the Kolmogorov-Smirnov test, for the Ret-He comparison the Mann-Whitney test was used. The test results were significant if the p-value <0.05.

Approval for ethical eligibility was obtained from the Health Research Ethics Commission, Faculty of Medicine, Hasanuddin University with number 370/UN6.4.5.31/PP36/2021.

RESULTS AND DISCUSSIONS

A total of 66 blood donors were divided into 33 regular blood donors and 33 non-routine blood donors, with more males (89.4%) than females (10.6%). According to the 2009 MOH age category, most of the subjects were in the 36-45 years old age group (30.3%), with mostly type B blood (33.3%) (Table 1).

Table 1. Characteristics of blood donors

Variable	n=66	%
Gender		
Male	59	89.4
Female	7	10.6
Age		
19-25 years old	13	19.7
26-35 years old	18	27.3
36-45 years old	20	30.3
46-56 years old	15	22.7
Blood type:		
A	18	27.3
B	22	33.3
AB	5	7.6
O	21	31.8
Type of donor		
Non-routine donor	33	50
Routine donor	33	50

Source: Primary data

Ret-He data normality test for routine and non-routine blood donors using the Kolmogorov-Smirnov test found that the Ret-He data distribution was not normally distributed.

Table 2. Comparison of Ret-He to donors

Type of Donors	n	Min	Max	Median	Mean	SD	p*
Routine	33	29.0	37.4	33.8	33.6	2.4	0.008
Non-routine	33	30.3	38.3	35.1	35.0	2.0	

*Mann-Whitney test

Ret-He comparison test by type of donor showed that the mean Ret-He in routine blood donors (33.6 pg) was lower than in non-routine blood donors (35 pg) (Table 2). The Mann-Whitney test showed a significant difference between routine and non-routine blood donors with p=0.008 (Figure 1).

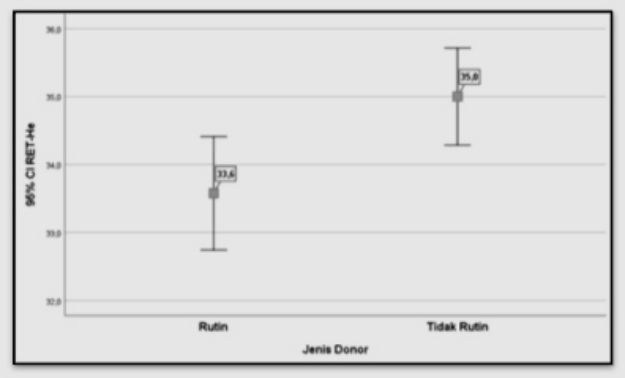

Figure 1. Ret-He comparative test according to the type of donor

The Ret-He comparison test according to donor type and gender (Table 3) found that the mean Ret-He in males was significantly lower in routine donors compared to non-routine donors, namely 33.6 compared to 35.2 with a value of p=0.004.

The mean Ret-He in females was found to be lower in routine donors than in non-routine donors, but not statistically significant (p=0.593).

Mean Ret-He was found to be lower in routine donors than in non-routine donors in all age groups, but all were not statistically significant with a p>0.05 (Table 4).

Table 5 comparison of Ret-He according to donor type and blood type, mean Ret-He was found to be lower in routine donors than in non-routine donors for all blood groups, but none were statistically significant with a p>0.05.

Blood donors are generally considered to be very healthy individuals because the donor undergoes a

Table 3. Comparisons of Ret-He according to the type of donor and gender

Gender	Type of Donor	n	Min	Max	Median	Mean	SD	p*
Male	Routine	30	29.0	37.4	33.9	33.6	2.2	0.004
	Non-routine	29	30.3	38.3	35,1	35.2	1.8	
Female	Routine	3	29.5	37.3	32.9	33.2	3.9	0.593
	Non-routine	4	30.5	38.3	33.3	33.9	3.3	

*Mann-Whitney test

Table 4. Comparison of Ret-He by donor type and age

Age Group	Type of Donor	n	Min	Max	Median	Mean	SD	p*
19-25 y.o	Routine	3	30.0	35.1	34.7	33.3	2.8	0.349
	Non-routine	10	30.4	37.1	34.8	34.3	2.2	
26-35 y.o	Routine	11	29.0	37.3	33.8	33.5	2.6	0.341
	Non-routine	7	30.3	36.5	35.1	34.4	2.1	
36-45 y.o	Routine	6	31.0	37.4	34.3	34.5	2.4	0.161
	Non-routine	14	32.4	38.3	35.4	35.8	1.8	
46-56 y.o	Routine	13	29.3	36.6	32.9	33.3	2.2	0.308
	Non-routine	2	33.8	36.0	34.9	34.9	1.6	

*Mann-Whitney test

Table 5. Comparison of Ret-He by type of donor and blood type

Blood Type	Type of Donor	n	Min	Max	Median	Mean	SD	p*
Type A	Routine	12	29.3	37.4	34.8	34.0	2.9	0.512
	Non-routine	6	30.5	38.3	34.7	35.2	2.9	
Type B	Routine	13	30.0	36.8	34.0	33.6	2.1	0.102
	Non-routine	9	32.4	38.2	35.5	35.2	1.6	
Type AB	Routine	3	29.0	33.3	32.5	31.6	2.3	0.083
	Non-routine	2	34.8	35.2	35.0	35.0	0.3	
Type O	Routine	5	31.8	35.5	33.5	33.6	1.5	0.116
	Non-routine	16	30.3	38.0	35.1	34.8	2.1	

*Mann-Whitney test

medical assessment before donating blood. In a total of 66 blood samples from 33 non-routine blood donors and 33 regular blood donors, the characteristics of blood donors were found to be more male than female donors, namely 89.4%. Most prospective female donors do not pass the selection for hemoglobin examination with an average value below 12.5 gr/dL. According to Tiwari *et al*., the number of female donors is very small because the tendency of anemia is more frequent in females.[7] According to Palokangas *et al*. assessing low ferritin levels, which is associated with worsening health in males that were regular blood donors, ferritin levels were found to show iron deficiency (ferritin level <15 µg/l) in 5% of males, 10% of female after menopause and 21% in the female before menopause from 1416 participants.[8] This study subjects were mostly in the 36-45 years old age group and had type B blood type.

Ret-He levels in routine blood donors were significantly lower than those in non-routine blood donors, this was because routine blood donors donated blood more frequently and regularly, thereby affecting the iron reserves in the donor's body. This study shows that the Ret-He level of routine blood donors is lower than that of non-routine blood donors but still within the normal Ret-He value (normal value: 30.2 pg-36.7 pg).[9] An increase in the frequency of blood donation reduces iron stores.[10-13] Donors who donate blood more than 4 times or more a year, experience depletion of iron stores, repeated blood donations have a positive effect on the donor's hematological parameters and biochemical biomarkers. Ferritin, one of the parameters that asses the depletion of iron reserves in normal conditions, can assess iron depletion before Iron-Deficient Erythropoiesis (IDE).[14] The interval for donating blood is about 8 weeks (56 days) for males and the minimum interval for females is 84 to 120 days.[15] According to Lee *et al*. study it is necessary to replace iron by consuming iron supplements in the Korean population based on the frequency of blood donation, which causes iron depletion. This can save health costs and is beneficial for blood donors.[16] Ret-He levels in routine blood donors decreased in this study, so appropriate iron supplementation must be considered in routine blood donors after donating blood because iron supplementation after blood donation only takes 3-5 days. In the HEIRS study, donors received iron supplements (37.5 mg) for 24 weeks, and total body iron returned to baseline the fastest in donors with low iron stores. In the STRIDE study, two doses of ferrous gluconate (19 mg and 38 mg) similarly increased iron depletion. Iron supplementation does not guarantee that donor iron will always be recovered, but it is the most effective intervention. Donors who consume iron will certainly increase iron stores better than just consuming iron from food alone.[14]

Iron deficiency anemia is often found in routine blood donors, currently only hemoglobin is used to screen blood donors in blood banks. Hemoglobin measurement alone will not detect blood donors with iron deficiency, so many blood donors receive blood with insufficient iron reserves but normal Hb values. According to research by Thomas *et al*. comparing routine blood donors with first-time blood donors, it was found that serum ferritin, serum Fe, Total Iron Binding Capacity (TIBC), transferrin saturation, and Complete Blood Count (CBC) levels in routine blood donors were significantly lower than first-time blood donors.[12]

Comparison of Ret-He according to donor type and gender was found to be significantly different in males, the sample of this study had more males than females according to Hindawi *et al*. It was found that 6.2% of females donated blood in Middle Eastern countries of the population studied, the difference in the number of males and females is of concern to researchers and specialists.[11]

Some of the reasons reported by females to explain this phenomenon included feeling inadequate to donate, not knowing the donation system, living far from a donation site, fear of worsening health with active menstrual bleeding, fear of increasing the likelihood of anemia, overload with household chores, fear of needles injections and fainting and fear of contracting an infection. Education and information about blood donation are needed, especially for females.[11] According to Armstrong, males usually donate blood more often than females, because they usually have larger stores of iron and lower iron needs compared to females of fertile age. Females are at greater risk of experiencing iron depletion due to iron loss during menstruation and pregnancy.[15]

Routine donors' Ret-He levels were lower than non-routine donors in all age groups but not statistically significant. There was no relationship

found between the type of donor and the age of the donor. Likewise, the Ret-He comparison between blood type and donor type was not significantly significant.

The limitation of this study is the need for another parameter to compare with Ret-He to see the depletion of iron storage and to see whether Ret-He decrease in routine blood donors depicts a decrease in iron reserve. A cohort study is needed to assess the extent to which Ret-He levels in routine blood donors fall below the normal range.

CONCLUSIONS AND SUGGESTIONS

It can be concluded that Ret-He levels in routine blood donors are lower than in non-routine blood donors, but still within normal limits. Further research should be carried out with other markers to compare with the results of this study.

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