Correlation Among Reticulocyte Hemoglobin, Immature Reticulocyte Fraction and Transferrin Saturation in Adult Patients with Iron-Deficiency Anemia

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Abstract

Iron deficiency anemia (IDA) is one of the most common forms of anemia with up to 80% of the world population suffering from it. Current widely used methods to determine IDA are hematology assay and transferrin saturation (TSAT). These methods have limitations such as they required two different specimens and could be affected by inflammation. Novel parameters such as reticulocyte hemoglobin (RET-He) could be used to measure iron reserve in bone marrow, whereas erythropoiesis is judged by immature reticulocyte fraction (IRF). Both parameters could be measured using one blood specimen. The aim of this study is to analyze the correlation between RET-He, IRF and TSAT in patients with IDA.

Keywords: iron deficiency anemia; immature reticulocyte fraction; reticulocyte hemoglobin; transferrin saturation

1. Introduction

Anemia is a condition of which the red blood cells or hemoglobin concentration in the red blood cell is lower than normal according to age group. The World Health Organization (WHO) defines anemia as hemoglobin concentration <13 g/dL for male above 15 years old, <12 g/dL for non-pregnant women above 15 years old, and <11 g/dL for pregnant women. World prevalence of anemia is 1.62 billion people (24.8% of all world population). [1]

Iron deficiency anemia (IDA) is one of the most common forms of anemia. It is approximated that 80% of the world population had IDA, and 30% of them had chronic IDA. Iron deficiency anemia could be caused by inadequate iron intake and/or iron malabsorption and gastrointestinal bleeding. Iron deficiency anemia could cause disturbance in growth and development and hinder daily activities. Laboratory examination plays an important role in diagnosis and therapy follow up in IDA. [2-4]

The current gold standard in diagnosing IDA is hemosiderin analysis from bone marrow with Perl's Prussian blue dye, but this method is highly invasive, comes with higher risk and only can be done by a highly trained laboratory analyst. Hemosiderin examination could be substituted with less invasive methods such as peripheral blood examination and iron status markers. Hematology examination is used to establish anemia diagnosis with cut-off value of hemoglobin <12.41 mg/dL for male and <11.92 mg/dL for female. Iron status examination is used for determining iron sufficiency, with diagnosis criteria involving low serum iron

concentration (<7,1 µg/L), low ferritin concentration (<30 ng/L) and high total iron binding capacity (TIBC) (>13.1 µmol/L). Transferrin saturation is a ratio as a result of calculation from serum iron concentration divided by TIBC. Hallak et al. stated that TSAT <16% could confirm IDA. This cut-off value is in accordance with the study of Molliner et al. in 2017. [3,5-7]

Hematology parameters and iron status have few weaknesses, such as needing 2 different samples: whole blood in EDTA tube and serum. Serum iron, TIBC, ferritin, TSAT examination could also be influenced by hemolytic, icteric and lipemic samples to a certain degree. Interleukin-6 secreted by macrophage and neutrophil could increase production of hepcidin. Higher hepcidin concentration could increase expression of ferritin and decrease ferroportin, thus limiting and sequestering iron from circulation and storing them in the form of ferritin. The serum iron concentration will decrease in inflammation and in the long term could cause anemia. [8-11]

Reticulocyte index parameters could be used as replacements for iron status and could be analyzed alongside hematology using the same sample. Reticulocyte hemoglobin (RET-He) is the result of measurement of hemoglobin content in reticulocytes. Reticulocytes are the first order in erythropoiesis after erythroblast releases its nucleus and migrates to peripheral blood circulation. Reticulocyte is present in peripheral blood earlier than erythrocyte; thus RET-He could give an earlier view of iron status in anemia than hemoglobin. [12]

Immature reticulocyte fraction (IRF) is an early marker of regeneration in erythropoiesis. Immature reticulocyte fraction could increase earlier than erythrocyte and hemoglobin and could show the bone marrow's response toward anemia in its pathophysiology as well as response of therapy.13,14

The aim of this study is to determine the correlation between TSAT and RET-He, TSAT and IRF, and RET-He and IRF in patients with IDA in Dr. Hasan Sadikin General Hospital.

2. Materials and Methods

This is a cross-sectional observational correlative study. All data were collected retrospectively using secondary data from medical records and laboratory results from laboratory and hospital information systems. The population of this study is all the medical records of adult patients with IDA (ICD-10 code D50) in the year 2019 which are 330 subjects.

The subjects of this study are patients with IDA diagnosis that have their iron status (serum iron, TIBC) and hematology (including RET-He and IRF) checked in Dr. Hasan Sadikin General Hospital in the period of January up to December 2019.

Inclusion criterion in this study is adult patients with IDA (TSAT <16%). Exclusion criterion in this study is patients with incomplete medical records and laboratory results. Correlative statistics analyses were done using SPSS 25 with Pearson formula if the data were normally distributed, and Spearman rank if the data were not normally distributed.

3. Results

There were 35 out of 330 (10.6%) subjects with IDA that fulfill the inclusion criteria. Characteristics of the subjects were described in Table 1 below.

Table 1. Characteristics of Included Subjects

Characteristics	n	%
Sex		
Male	13	37.1%
Female	22	62.9%
Age		
15 – 24	7	20.0%
25 – 44	19	54.3%
45 – 64	9	25.7%
RET-He		
Low (<32,1 g/dL)	35	100%
Normal (32,1 – 38,8 g/dL)	0	0%
IRF		
Normal (1,6 – 10,5 %)	1	2.9%
High (>10,5 %)	34	97.1%

Majority of the subjects were female (62.9%) with age of 25 to 44 years old (54.3%). All subjects (100%) have low RET-He value, with 97.14% of the subjects having high IRF. The normality of the TSAT, RET-He, IRF data were tested using the Shapiro-Wilk test with the result of normal distribution for RET-He and IRF and abnormal distribution for TSAT data. The data of RET-He and IRF were presented as mean ± standard deviation, and TSAT as median (minimum, maximum) in Table 2.

Table 2. Transferrin Saturation, RET-He and IRF Results

Parameters	Mean ± SD	Median (Min, Max)
Saturasi Transferin	-	4.7% (1%, 14%)
RET-He	18.9 ± 4.4	-
IRF	25.9 ± 9.9	-

The correlation between TSAT and RET-He, TSAT and IRF, and RET-He and IRF were displayed in Table 3, 4, and 5.

Table 3. Correlation Between TSAT and RET-He

	RET-He	
	ρ	**P**
TSAT	0.513	0.002

Table 4. Correlation Between TSAT and IRF

	IRF	
	ρ	**P**
TSAT	0.192	0.270

Table 5. Correlation Between RET-He and IRF

	IRF	
	r	**P**
RET-He	0.080	0.649

There were moderate and significant correlation between TSAT and RET-He (ρ=0.513, P=0.002), but no significant correlation between TSAT and IRF (ρ=0.192, P=0.270) and between RET-He and IRF (r=0.080, P=0.649).

4. Discussion

Transferrin saturation and RET-He were found relatively low in all subjects with moderate but significant correlation. These results were in accordance with those in studies by Mast et al., Davidkova et al., Kaneko et al., and M. Rudiyansah et al. reveal that RET-He concentration will decrease following depletion of iron reserves as demonstrated by low TSAT. Hemoglobinization of immature erythrocyte depends on iron reserves, thus decrease of iron could impair hemoglobin synthesis in the erythropoiesis process. [15-18]

Majority of the subjects (97.14%) show an increase of IRF that reflects higher erythropoiesis as a response of IDA. This result is similar to the study of Choi et al that showed a higher population of middle and high-fluorescence reticulocytes in patients with low serum iron. [19]

Even though there were increases in IRF value in almost all subjects, there was no significant correlation between IRF and TSAT or RET-He. This could be caused by the decreasing serum iron not having a direct impact on erythropoietin. The decreasing serum iron will cause anemia and lower the oxygen concentration in tissues. Low oxygen concentration could trigger erythropoietin gene expression in the kidney thus increasing erythropoietin secretion. Increased erythropoietin could promote erythroid progenitor cell division and maturation and also restrain erythroblast apoptosis in the bone marrow. This whole process from the increase of erythropoietin until release of young reticulocytes into peripheral blood circulation could take 1 – 2 weeks.

The acquired IRF value in the time of low TSAT and RET-He reflects erythropoiesis process up to 1 – 2 weeks before. [20-22]

This study has a few limitations such as not every patient with IDA had prior RET-HE and IRF results, resulting in small sample size. This could explain the non-existence of correlation between TSAT and RET-He toward IRF. This study also did not regard the hemoglobin level which could vary between samples as an effect of blood transfusion, thus could affect the rate of erythropoiesis. Further research is needed to assess the correlation of TSAT and RET-He toward IRF with bigger sample size.

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Conflict of Interest Statement

The authors have no potential conflict of interest regarding to this work.

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