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*by Yustisia Amalia*

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**Submission date:** 08-Nov-2021 10:09PM (UTC-0600)

**Submission ID:** 1697419271

**File name:** document.pdf (227.64K)

**Word count:** 3156

**Character count:** 17089

## Difference of Ret Level In Thalassemia $\beta$ Minor And Iron Deficiency Anemia

Yustisia Amalia\*

Universitas Dr. Soetomo Surabaya, Indonesia

\* [yusti.amalia@yahoo.com](mailto:yusti.amalia@yahoo.com)

### ABSTRACT

The most common diseases found with peripheral blood morphological features of hypochromic microcytic anemia are iron deficiency anemia and thalassemia. Hypochromic microcytosis is a morphological description of red blood cells with MCV values smaller than normal ( $<80\text{fl}$ ) and MCH smaller than normal values ( $<27\text{pg}$ ). This morphological picture can be found in the condition of iron deficiency anemia and thalassemia. There are several markers for the assessment of Hb content in reticulocytes, including Ret-He. Ret-He, which can be measured by the latest automated hematological analysis, is considered to reflect the iron content in reticulocytes. This research is an observational analytic study using case control measurement method, during July to September 2019 at the Surabaya Medical Centre Hospital. The population was students who were admitted to the University of Dr. Soetomo who came for complete blood count examination. The subjects were students with MCV  $<80\text{ fl}$ , MCH  $<27\text{ pg}$ . Subjects who met the inclusion criteria then continued with the calculation of the Mentzer Index and RDW Index, ferritin serum examination using the ECLIA method, hemoglobin electrophoresis examination with micro capillary electrophoresis. By consecutive sampling, 42 samples were obtained, of which 21 subjects diagnosed with iron deficiency anemia and 21 subjects diagnosed with beta thalassemia minor were examined for their Ret-He levels using the flow cytometric method which were then analyzed statistically. From 21 iron deficiency anemia patients it was found that the average Ret-He value was 30.64 (6.08) pg and from 21 patients with beta minor thalassemia it was found that the average Ret-He value was 25.63 (6.72) pg. The results of the unpaired t-test for both groups obtained  $p = 0.016$ . By using the ROC curve obtained the Ret-He cut-off value in distinguishing cases of iron deficiency anemia with thalassemia was 27.30 pg with a sensitivity of 90.5% and specificity of 71.4%.

From the results of the study concluded that there was a significant difference between the levels of Ret-He in patients with iron deficiency anemia with thalassemia beta minor patients with a cut-off value of 27.30pg

**Keywords:** Iron Deficiency Anemia, Thalassemia, Ret-He

Received October 1, 2020; Revised October 14, 2020; Accepted October 29, 2020



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## BACKGROUND

The most common diseases found with peripheral blood morphological features of hypochromic microcytic anemia are iron deficiency anemia and thalassemia. Hypochromic microcytic is a morphological description of red blood cells with MCV values smaller than normal ( $<80\text{fl}$ ) and MCH smaller than normal values ( $<27\text{pg}$ ).<sup>1,2,3</sup> Iron deficiency anemia is an anemia caused by low iron for erythropoiesis<sup>1,4,5,6</sup>, while Thalassemia is genetic disorder which cause decrease globin chain synthesis<sup>7,8,9</sup>.

Thalassemia is widespread in areas in mainland China in border like Muangthai, Laos, Kamboja with a frequency of 50-60% and also spread in southeast area with lower frequency. In Indonesia, expected in 3-5%, same as Malaysia and Singapura<sup>7,9</sup>.

Reticulocyte Hemoglobin measure reflects a dynamic erythropoiesis process in bone marrow<sup>10</sup>. Ret-He is an iron index and reflect availability of cellular iron<sup>10</sup>. Ret-He got a collaboration with iron deficiency and a sign for iron deficiency in child and baby, adult blood donation, geriatric, pregnant woman and chronic kidney disease patient in hemodialysis<sup>11,12,13</sup>. erythrocytes and reticulocytes can be used to help differentiate thalassemia and iron deficiency anemia<sup>14</sup>.

This research to differentiate the value of Ret He in thalassemia and iron deficiency anemia in RS Surabaya Medical Center Surabaya.

## METHODS

This research is an analytical research with design case control. One-time observation to obtain data on Ret-He levels from minor beta thalassemia and iron deficiency anemia patients at Surabaya Medical Center Surabaya Hospital. The population is a student accepted at Dr. Soetomo University who comes for a complete blood test.

Patients who meet inclusion criteria such as 1) hypochromic microcytics (MVC  $< 80\text{ fl}$ , MCH  $< 27\text{pg}$ ) 2) minor beta Thalassemia patients who have confirmed laboratory examination 3) iron deficiency anemia patients who have confirmed laboratory examination 4) maximum age 18 years 5) patients willing to be research samples and sign informed consent. The subject meets the inclusion criteria followed by the calculation of Mentzer Index and RDW Index, examination of serum Ferritin by ECLIA method, examination of Electrophoresis Hemoglobin with micro capillary electrophoresis. Consecutive sampling of 42 samples, of which 21 subjects were diagnosed with iron deficiency anemia and 21 subjects with minor beta thalassemia diagnoses. Patients who had had blood transfusions in the last 3 months, received iron supplementation, and suffered from other systemic diseases were exclusivity from this study.

Edta venous blood samples of 42 patients were examined from July 2019 to September 2019. Checks are performed with Sysmex XN-1000 which has been done quality control before. Complete blood analysis is done by automatic cell counting with flowcytometry principle. The data taken is in the form of complete blood and Ret He levels.

Analysis of research data was conducted using SPSS by performing bivariate analysis using unpaired T for data with normal distribution and Mann Whitney test for data that was not distributed normally. Receiver-operating characteristic (ROC) curves are performed to determine the cut point of Ret-He levels in distinguishing cases of Iron Deficiency Anemia with Thalassemia Beta minor. P value  $< 0.05$  is said to be statistically meaningful

## RESULTS

The data analysis was conducted on 42 patients with a male gender of 4 (9.5%) with an average age of 17.95 years standard deviation of 0.49 and the median age is 18 years with

the lowest age is 16 years and the highest age is 18 years. The characteristics of the research sample can be seen in the table below.

**Table 1 Sample Characteristic**

Variable	N	%	Mean(SD)	Median(Min-Max)
Age (years)			17,95(0,49)	18(16-18)
Sex				
Male	4	9,5		
Female	38	90,5		
Ethnic				
Jawa	10	23,8		
Toba	9	21,4		
Melayu	8	19,0		
Mandailing	5	11,9		
Tionghoa	3	7,1		
Karo	2	4,8		
Simalungun	2	4,8		
Banjar	1	2,4		
Nias	1	2,4		
Toraja	1	2,4		

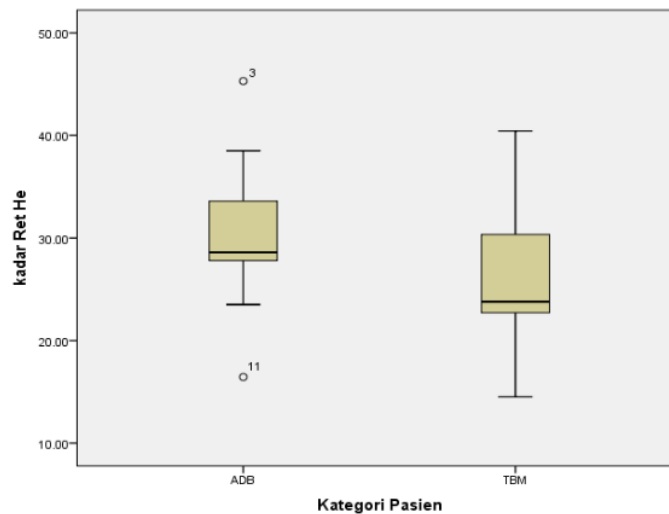
Median hemoglobin (Hb) levels of iron deficiency anemia and thalassemia were found to be 9.60% and 9.80% (p 0.0037). Median MCV value and mentzer index of minor beta thalassemia patients were lower than iron deficiency anemia (61.59; 12.30) while the median RDW value of thalassemia patients was higher (16.60).

**Tabel 2. Data on measurement of Hemoglobin levels, number of RBC, MCV value, RDW value, Mentzer Index, RDW Index, Ferrite Levels in Iron Deficiency Anemia patients and Minor Beta Thalassemia patients**

Variable	Iron Deficiency Anemia	Thalassemia Beta Minor	P Value
	Mean(SD)	Mean(SD)	
	Median(Min-Max)	Median(Min-Max)	
Hb value (gr %)	9,60(8,40-10,00)	9,80(8,20-10,70)	0,037**
RBC (juta/mm <sup>3</sup> )	3,77(3,56-3,96)	5,13(4,56-5,81)	<0,001**
MCV (fL)	74,09(2,68)	61,59(4,30)	<0,001*
RDW	12,60(11,50-14,20)	16,60(11,60-18,40)	<0,001**
Mentzer Index	19,80(18,30-21,10)	12,30(9,70-12,90)	<0,001**
RDW Index	324,76(29,55)	193,80(19,64)	<0,001*
Feritin (mcg/L)	8,00(3,00-10,00)	87,00(40,00-179,00)	<0,001**

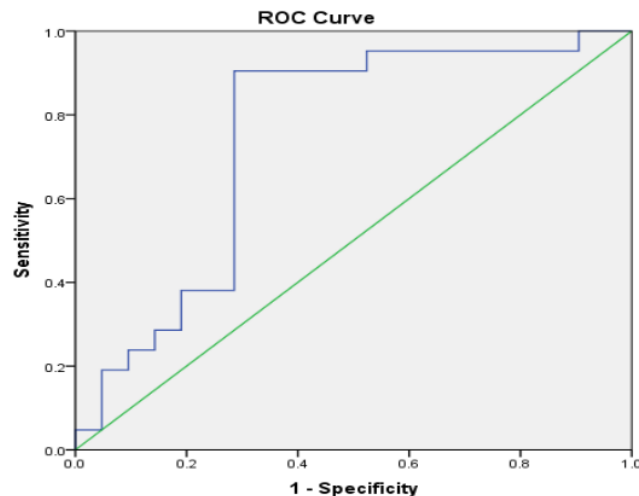
\*T- unpaired test\* \*Mann Whitney Test

There was a significant difference between Ret-He levels in iron deficiency anemia patients and minor beta thalassemia patients, where Ret-He levels in Thalassemia Beta Minor patients (25.63±6.72 pg) were lower compared to Ret-He levels in iron deficiency anemia patients (30.64±6.08 pg) [p 0.016]. Image 1



**Image 1. Comparison of Ret-He levels in iron deficiency anemia patients with minor beta thalassemia patients**

The value of Ret-He discrimination in distinguishing cases of iron deficiency anemia with minor beta thalassemia was assessed by the Receiver Operating Characteristic (ROC) method and found AUC 74.6%, with a confidence interval of 0.586 – 0.906 (IK 95%,  $p=0.006$ ) with moderate quality discrimination. From this analysis, the ret-he cut point value was 27.30 pg with a sensitivity of 90.5% and a specificity of 71.4%.



**Image 2 Receiver-operating characteristics curve analysis to assess Ret-He performance in distinguishing cases of iron deficiency anemia with minor beta thalassemia**



## DISCUSSION

This research is a case control study that aims to find out the role of Ret-He levels in distinguishing cases of iron deficiency anemia with beta minor thalassemia. In general, iron deposits are reduced or lost before people develop anemia. Therefore, diet and iron recycled erythrocytes must meet the demands for the production of erythrocytes. If iron loss continues, the newly produced erythrocytes will decrease hemoglobin, causing the amount of iron provided by erythrocytes to decrease. Unlike thalassemia, an increase in the amount of erythrocytes is not produced in a state of iron deficiency to compensate for the decrease in intracellular hemoglobin levels. For this reason, reticulocytosis usually does not exist. In the absence of major bleeding, iron deficiency anemia generally develops slowly for months or years<sup>15</sup>.

Thalassemia is a genetic blood disorder, caused when the body makes fewer healthy red blood cells and fewer hemoglobins than normal. Hemoglobin is a protein in red blood cells that carries oxygen to all tissues of the body. Hemoglobin usually consists of two  $\alpha$ - and two globins. The nature of thalassemia syndrome is associated with reduced or absence of synthesis of any of these two chains. The  $\alpha$ -thalassemia is lower than  $\beta$ -thalassemia and usually shows no symptoms<sup>16</sup>.

Most current hemoglobinopathy screening methods include high performance liquid chromatography (HPLC), hemoglobin electrophoresis, PCR mutation screening, and DNA testing. All of these methods result in higher project costs and require special instrumentation and trained technicians. In addition, currently various indexes and formulas of red blood cells (RBC) have been designed to distinguish between thalassemia and IDA and its sensitivity and specificity are presented in various articles<sup>16</sup>.

From the study conducted found significant differences between Hemoglobin levels, MCV values, RDW values, Mentzer Index and Ferrite levels in iron deficiency anemia patients with minor beta thalassemia patients. This is in line with previous research that conducted research on the use of new indexes for hematological parameters to distinguish cases of iron deficiency anemia with cases of thalassemia beta minor<sup>17</sup>. The results found significant differences between, hemoglobin levels, MCV values, MCH values, ferritin levels between patients with iron deficiency anemia and minor beta thalassemia patients with a  $p < 0.001$  value. However, there was no significant difference between the RDW value of patients with iron deficiency anemia and minor beta thalassemia patients with a value of  $p = 0.94$ <sup>17</sup>.

From the study, there was a significant difference between Ret-He levels in iron deficiency anemia patients and minor beta thalassemia patients with a value of  $p = 0.016$ , where Ret-He levels in minor beta thalassemia patients were lower than those with iron deficiency anemia. This is in line with research on efficacy from the use of new parameters in distinguishing cases of minor  $\beta$ -thalassemia, iron deficiency anemia, megaloblastic anemia and aplastic anemia<sup>18</sup>. The study found that Ret-He levels in minor beta thalassemia patients were lower ( $20.92 \pm 1.5$  pg) compared to patients with iron deficiency anemia ( $21.0 \pm 9.1$ ). This is because Ret-He exhibits recent changes in the synthesis of hemoglobin in reticulocytes. Ret He measurement is a direct assessment of the merging of iron in haemoglobin. This parameter is highly sensitive and specific due to the short and meaningful life span of reticulocytes for early diagnosis of iron deficiency erythrooiesis<sup>18</sup>.

Reticulocytes parameters are better for detecting iron-deficient erythrooiesis than red blood cell indexes. Reticulocytic maturation assessment is beneficial in establishing an anemia mechanism and effective assessment of erythrooiesis<sup>19</sup>. Reticulocytes are released

from the marrow 18 to 36 hours before their last maturation; they provide a real-time assessment of the functional status of erythropoiesis<sup>20</sup>.

Ret-He represents the latest change in hemoglobin synthesis in reticulocytes. Ret He measurement is a direct assessment of the merging of iron in hemoglobin<sup>14</sup>. This parameter is very sensitive and specific due to the short life span of reticulocytes and is very meaningful for early diagnosis of iron-deficient erythropoiesis<sup>21</sup>.

The discrimination performance of Ret-He Levels in distinguishing cases of iron deficiency anemia was presented with an AUC value of 74.6% (IK 95% = 0.586 – 0.906) indicating moderate quality discrimination. In this study obtained a cut point Ret-He in distinguishing cases of iron deficiency anemia with beta minor thalassemia is 27.30 pg. The results of this study show differences with previous research on the parameters of hemoglobin reticulocytes equivalent in iron deficiency anemia patients<sup>22</sup>. From the study obtained a cut off value of 28.5 pg to diagnose iron deficiency anemia patients. This difference lies in the expected end result and the population used. This led to this study having a lower cut point value of Ret-He levels than other studies.

## CONCLUSION

There was a significant difference in ret-he levels between patients with iron deficiency anemia and minor beta thalassemia patients with a value of  $p = 0.016$ . The cut off value of Hemoglobin reticulocytes in distinguishing cases of iron deficiency anemia with minor beta thalassemia patients is 27.85 pg with a sensitivity of 90.5% and specificity of 71.4%. Hemoglobin reticulocytes should be included in a complete hematological examination. More research is needed on Ret-He for its application with other diseases.

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