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RESEARCH

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## **ERYTHROCYTE INDICES TO DIFFERENTIATE IRON DEFICIENCY ANEMIA FROM $\beta$ TRAIT THALASSEMIA**

*(Indeks Eritrosit untuk Membedakan Anemia Defisiensi Besi dengan Thalassemia  $\beta$  Trait)*

**Yohanes Salim<sup>1</sup>, Ninik Sukartini<sup>1</sup>, Arini Setiawati<sup>2</sup>**

### **ABSTRAK**

Anemia defisiensi zat besi dan thalassemia  $\beta$  trait merupakan penyebab tersering anemia mikrositik hipokrom di Indonesia. Kedua penyakit tersebut sulit dibedakan hanya dengan pemeriksaan hematologi, oleh karena itu diperlukan pemeriksaan tambahan seperti feritin dan analisis hemoglobin. Banyak penelitian yang membedakan kedua penyakit tersebut dengan indeks eritrosit. Berbagai indeks eritrosit memiliki nilai diagnostik yang berbeda di setiap negara dan belum ada data di Indonesia. Penelitian ini melakukan uji diagnostik Indeks Mentzer, RDW, Green-King, Sirdah dan mencari nilai cut-off baru yang dapat memberikan nilai diagnostik lebih baik. Penelitian terdiri dari 98 subjek definitif anemia defisiensi besi dan 80 subjek thalassemia  $\beta$  trait. Indeks Mentzer didapatkan kepekaan 83,6%, kekhasan 66,2%, NPP 75,2%, NPN 76,8% dan Indeks RDW mempunyai kepekaan 91,8%, kekhasan 75%, NPP 81,8%, NPN 88,2%. Nilai diagnostik Indeks Green-King didapat kepekaan 96,9%, kekhasan 67,5%, NPP 78,5%, NPN 94,7%, sedangkan Indeks Sirdah adalah kepekaan 92,8%, kekhasan 58,7%, NPP 73,3%, NPN 87,0%. Nilai cut-off baru Indeks Mentzer adalah 13,44, RDWI 233,4, Green-King 75,06 dan Sirdah 32,52. Keempat Indeks eritrosit dapat diterapkan untuk orang Indonesia dengan Indeks Green-King sebagai indeks yang terbaik.

**Kata kunci:** Anemia defisiensi zat besi, thalassemia  $\beta$  trait, indeks Mentzer, RDW, Green-King, Sirdah

### **ABSTRACT**

Iron deficiency anemia and  $\beta$  trait thalassemia are the most common causes of microcytic hypochromic anemia in Indonesia. Differentiation between them is difficult when solely based on a hematologic examination; additional laboratory tests are required such as ferritin and hemoglobin analysis. However, not all laboratories can perform these tests. Many erythrocytes indices have been proposed to determine whether a blood sample is more suggestive for iron deficiency anemia or trait thalassemia. Unfortunately these indices have different diagnostic values in many countries and there is no data yet about the diagnostic value in Indonesia. This study performed diagnostic tests for Mentzer, RDW, Green-King and Sirdah Indices and developed a new cut-off point that could make a better diagnostic value. This study consisted of 98 subjects of iron deficiency anemia and 80 subjects of  $\beta$  trait thalassemia. Diagnostic values of Mentzer Index were sensitivity 83.6%, specificity 66.2%, PPV 75.2%, NPV 76.8%, while RDW Index had a sensitivity of 91.8%, specificity of 75%, PPV 81.8% and NPV 88.2%. Diagnostic values of Green-King Index were as follows, sensitivity 96.9%, specificity 67.5%, PPV 78.5%, NPV 94.7%, while diagnostic values of Sirdah Index showed a sensitivity of 92.8%, specificity of 58.7%, PPV 73.3% and NPV 87.0%. The new cut-off point of Mentzer, RDW, Green-King, and Sirdah Index were 13.44, 233.4, 75.06 and 32.52, respectively. All indices can be applied for Indonesian people, among which Green-King Index had the best diagnostic value.

**Key words:** Iron deficiency anemia,  $\beta$  trait thalassemia, Mentzer, Green-King, Sirdah

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## INTRODUCTION

Iron deficiency anemia and  $\beta$  trait thalassemia are the most common causes of hypochromic microcytic anemia found in developing countries and in countries with endemic thalassemia disease, such as Indonesia.<sup>1-5</sup> However, both are difficult to distinguish just by hematology examination.<sup>6</sup> Thus, additional laboratory tests, such as iron status and hemoglobin analysis are needed to distinguish although they require additional time and expense, as well as not all laboratories can perform the examination.<sup>7,8</sup>

Many researches actually have been conducted to distinguish these two diseases easily, fast, practically, simply and accurately using erythrocytes indices consisting of a formula and *cut-off* values for four decades. The formula of erythrocyte index involves a parameter that already exist on hematological examination using automated blood cell count. Unfortunately, erythrocyte indices have different diagnostic values in each country and in Indonesia there are still no data used to compare the diagnostic values of various erythrocyte indices at once. England, Shine-Lal and Rajabiani also have suggested to set *cut-off* values of each index in different populations.<sup>8,9</sup>

Therefore, this research aimed to obtain diagnostic values and *cut-off* values in order to provide better diagnostic values than the standard *cut-off* ones set by each previous respective index. Erythrocyte index studied in this research is a familiar index that has good diagnostic values in other countries, such as *Mentzer*, *Red Cell Distribution Width* (RDW), *Green-King* and *Sirdah*. Thus, the results of this research are expected to help clinicians in differentiating iron deficiency anemia from  $\beta$  trait thalassemia using erythrocyte index screening when there is limited analysis of hemoglobin and ferritin examination.

## METHODS

This research is a retrospective diagnostic test. Subjects in this research were patients with definitive iron deficiency anemia and  $\beta$  trait thalassemia undergoing several examinations complete hematology, hemoglobin electrophoresis, SI, UIBC, TIBC, transferrin saturation, and ferritin levels in the Department of Clinical Pathology, Dr. Cipto Mangunkusumo Hospital from August 2010 to July 2015. Subjects were of the age of over 12 months with some exclusion criteria for both groups, namely abnormal leukocyte count and abnormal absolute reticulocyte count. Another exclusion criterion for the group of iron-deficiency

anemia is low platelet count. Meanwhile, other exclusion criteria for the group of  $\beta$  trait thalassemia were abnormal platelet count, iron status, serum ferritin levels and low transferrin saturation.

Operational constraints for iron deficiency anemia, moreover, were determined based on Hb of <13 g/dL for adult males <12 g/dL for females, or less than the Hb reference value according to age; MCV of <82 fL for adults or less than the MCV reference value according to age; MCH of <27 pg for an adult or less than the MCH reference value according to age; serum ferritin level<sup>10</sup> of <30 ng/mL for males and <13 ng/mL for females and / or transferrin saturation <15%; as well as normal or decreased hemoglobin fraction, HbA<sub>2</sub>, without HbH fraction. Thalassemia  $\beta$  trait, on the other hand, was determined by a decrease in hemoglobin concentration, MCV and MCH. Like iron deficiency anemia, thalassemia  $\beta$  trait was also determined with serum ferritin level of >30 ng/mL for men, >13 ng/mL for women and/or transferrin saturation of >15%, with the results of hemoglobin analysis indicating  $\beta$  thalassemia trait, HbA<sub>2</sub> fractions from 3.8 to 7.0%.<sup>11</sup>

Next, categorical variable data were presented in percentages. Numerical variable data were tested using Kolmogorov-Smirnov test to determine the distribution of the data. If the normal distribution of data was presented in mean (standard deviation), and the abnormal distribution of data was presented in median and minimum-maximum value range.

The formula to get the index value of each subject is listed in Table 1. Having obtained the Index value, the subjects of deficiency anemia and  $\beta$ -thalassemia trait were separated based on the *cut-off* value as seen in Table 2 for Mentzer Index. Each index was tested diagnostically with 2×2 table to get sensitivity value, specificity value, Positive Predictive Value (PPV), Negative Predictive Value (NPV), Positive Likelihood Ratio (PLR) and Negative Likelihood Ratio (NLR). With ROC curve obtained by using SPSS, the *cut-off* value of new index then was determined to produce the most optimal diagnostic value.

## RESULTS AND DISCUSSION

Based on data from August 2010 to July 2015, the number of subjects in research met the criteria was 178 subjects, 98 of whom were categorized as iron deficiency anemia patients (55%), while 80 of whom were categorized as thalassemia  $\beta$  trait patients (45%). The subjects of this research had also been selected so that there was no combined diagnosis and/or

**Table 1.** Calculated index

Index	Formula*	Cut-off values
Mentzer	MCV/Erythrocyte count	>13 = Iron deficiency anemia <13 = $\beta$ Trait thalassemia
RDW	MCV $\times$ RDW/Erythrocyte count	>220 = Iron deficiency anemia <220 = $\beta$ Trait thalassemia
Green-King	MCV $\times$ MCV $\times$ RDW/(Hb $\times$ 100)	>65 = Iron deficiency anemia <65 = $\beta$ Trait thalassemia
Sirdah	MCV–Erythrocyte Count– (3 $\times$ Hb)	>27 = Iron deficiency anemia <27 = $\beta$ Trait thalassemia

\*MCV (fL), Erythrocyte Count ( $\times 10^6$ ), RDW (%), Hb (g/dL)

**Table 2.** Diagnostic test with Mentzer index on iron deficiency anemia

Mentzer index	Iron deficiency anemia	$\beta$ trait thalassemia	Total
>13	a	b	a+b
<13	c	d	c+d
Total	a+c	b+d	a+b+c+d

**Table 3.** Laboratory results of subjects with hypochromic microcytic anemia

Parameter	Iron deficiency anemia	$\beta$ thalassemia trait
Hemoglobin	8.36 (1.8)*	10.6 (7.4-12.4)**, mean 10.23
Hematocrit	28.23(4.89)*	32.90(22.58-39.12)**
Erythrocyte count	4.18 (0.71)*	5.24 (0.66)*
MCV	67.83 (7.71)*	61.87 (4.94)*
MCH	20.04 (3.38)*	19.83 (1.65)*
Leukocyte	7.72 (1.57)*	7.77 (1.77)*
Hemoglobins	321.5 (155-755)**, mean 347.5	286.61 (72)*
RDW-CV	18.58 (2.90)*	16.10 (14.1-27.6)**, mean 16.8
Reticulocyte	49.711 (25.254-93.958)**	69.988 (25.326-97.026)**
HbA	97.8 (96.7-98.7)**	94.3 (85.8-95.9)**
HbF	0 (0-0.4)**	0.4 (0-7.2)**
HbA2	2.2 (1.3-3.3)**	5.15 (0.57)*
Ferritin	5.7 (0.5-18.6)**	90.35 (17.7-2.058)**

\* Normal distribution of data presented in the mean (SD) \*\* Abnormal distribution of data presented in median (min-max)

no chronic anemia disease in accordance with the inclusion and exclusion criteria.

Furthermore, the laboratory results of complete hematology, hemoglobin fraction, and ferritin were shown in Table 2. All research subjects with hypochromic microcytic anemia were determined by a decrease in hemoglobin concentration, MCV and MCH. In Table 3, the mean of Hb levels in  $\beta$  thalassemia trait was higher than that of iron deficiency anemia. This was because the clinical spectrum of  $\beta$  thalassemia trait was from asymptomatic to intermedia, whereas

iron deficiency anemia depended on underlying causes,<sup>12</sup> so the obtained Hb level was derived from severe to mild iron deficiency anemia.

In iron deficiency anemia, the mean of MCV was higher than that of  $\beta$  thalassemia trait. This was due to iron deficiency anemia which had an erythrocyte spectrum from normocytic to microcytic volume in iron deficiency anemia. Considering the average lifespan erythrocytes of 120 days, erythrocytes were still normocytic at the age near 120 days before iron deficiency anemia occurred and were then mixed with

**Table 4.** Diagnostic values of Mentzer index, RDW, Green-King and Sirdah

Index	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	PLR	NLR
Mentzer	83.6	66.2	75.2	76.8	2.4	0.2
RDW	91.8	75.0	81.8	88.2	3.6	0.1
Green-King	96.9	67.5	78.5	94.7	2.9	0.04
Sirdah	92.8	58.7	73.3	87.0	2.2	0.1

microcytic erythrocytes that had already experienced iron deficiency anemia. In  $\beta$  thalassemia trait, erythrocyte volume tended to be stable microcytic, so MCV mean was not affected by normal erythrocyte volume as in iron deficiency anemia. Consequently, RDW-CV in  $\beta$  thalassemia trait was higher than that in iron deficiency anemia. Table 4 summarized the diagnostic values of Mentzer index, RDW, Green-King, and Sirdah.

The diagnostic values obtained in this research were not exactly the same as those in studies in other countries. It could be due to differences in the subjects studied. This research used data of subjects with definitive iron deficiency anemia and  $\beta$ -thalassemia trait, both from Outpatient and Inpatient clinics, so they are considered to have a diverse clinical spectrum. In studies in several other countries, the subjects used were mostly patients undergoing *medical check-up* or clinically healthy.<sup>3-6, 8, 13</sup> so they obtained were 'typical' subjects. Nonetheless, the trend of the diagnostic values in this research was still quite similar because it showed results of sensitivity and specificity obtained of approximately 65–90%.

Sensitivity of Mentzer index in this research, was similar to the sensitivity in researches conducted by Vehapoglu<sup>3</sup> in Turkey (82.3%), Moghaddam<sup>6</sup> in Iran (82%) and Niazi<sup>14</sup> in Pakistan (81%) although the specificity in this research was different (respectively 98.7, 72 and 89 %). The differences in specificity was due to differences in population characteristics of  $\beta$  thalassemia trait. Mentzer index formula showed *MCV divided by erythrocyte count*. In Moghaddam's research<sup>6</sup>, mean MCV was 61 fL and erythrocyte count was 7.1 (in  $10^6/\mu\text{L}$ ), so the average value of the index was 8.59. Meanwhile, in this research, the mean MCV was 61.8, and erythrocyte count was 5.24 fL (in  $10^6/\mu\text{L}$ ), so the mean value of the index was 11.79. This difference could be seen in the erythrocyte count in Moghaddam's<sup>6</sup> research which was larger. The larger erythrocyte count as the denominator resulted in a smaller index value than that in this research. The index values of  $\beta$  thalassemia trait subjects in this research tended to be more at the value of >13

compared to those in Moghaddam's research<sup>6</sup>, so a decrease in specificity occurred.

The sensitivity in RDW index in this research, was similar to the sensitivity in researches conducted by Matos<sup>15</sup> in Brazil (93.8%) and Huang<sup>5</sup> in Taiwan (93%) although their specificity was different from this research (80.9 and 81%). The difference in specificity was due to differences in population characteristics of  $\beta$  thalassemia trait. The formula of RDW index showed *MCVxRDW/erythrocyte count*.  $\beta$  thalassemia trait in the research of Matos<sup>15</sup>, showed a mean MCV of 63.7 fl, RDW 15.8%, and erythrocyte count 5.47 (in  $10^6/\mu\text{L}$ ), so the mean value of the index was 181.9. In the research conducted by Huang<sup>5</sup> the mean MCV was 62.4 fl, RDW 15.6% and erythrocyte count 6.14 (in  $10^6/\mu\text{L}$ ), so the mean value of the index was 158.5. Meanwhile, in this research, the mean MCV was 61.8 fL, RDW 16.8% and erythrocyte count 5.24 (in  $10^6/\mu\text{L}$ ), so the mean value of the index was 198.1. The difference of this research was that compared to Matos<sup>15</sup> and Huang<sup>5</sup> the mean MCV was the smallest one, RDW was the greatest one and the erythrocyte count was the smallest one. In other words, if the MCV value as the numerator was small it will generate a small index value, but if the RDW as the numerator was large and erythrocyte count as the denominator was small it can generate a large index value. This means that the mean of the index in this research was greater than that in those two studies. Therefore,  $\beta$  thalassemia trait subjects in this research tended to be more likely to have a value of >220 compared to those in the researches of Matos<sup>15</sup> and Huang<sup>5</sup> resulting in a decrease in specificity.

In addition, sensitivity in Green-King index in this research was similar to sensitivity in some researches conducted by Keikhaei<sup>9</sup> in Iran (94.3%) and Huang<sup>5</sup> in Taiwan (94%) even though the specificity in those researches was different from that in this research (90.9 and 83%). This difference in specificity was due to differences in population characteristics of  $\beta$  thalassemia trait. The formula of Green-King index showed *MCVxMCVxRDW/(Hbx100)*. In the research conducted by Huang<sup>5</sup>, the mean MCV was 62.4 fl,

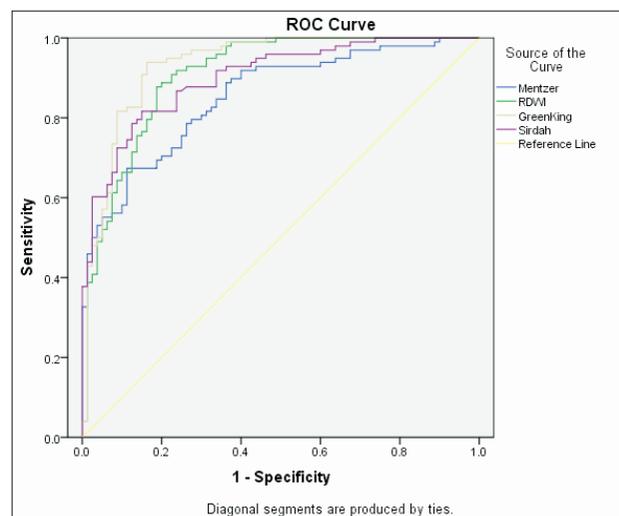
RDW 15.6%, and hemoglobin 12.2 g/dL, so the average value of the index was 49.7. In this research, on the other hand, the mean MCV was 61.87 fL, RDW 16.1% and hemoglobin 10.6 g/dL, so the average value of the index was 58.14. It indicated that if the MCV value, as the numerator is small; it will generate a small index value, but large RDW as the numerator and a small Hb as the denominator can generate a great index value. Therefore, the mean value of the index in this research was greater than that in the research conducted by Huang.<sup>5</sup> This means that  $\beta$  thalassemia trait subjects in this research tended to be more likely have a value of  $>65$  compared to those in Huang<sup>5</sup> resulting in a decrease in specificity.

The sensitivity in Sirdah index in this research was similar to sensitivity in researches conducted by Matos<sup>15</sup> in Brazil (94.1%) and Keikhaei<sup>9</sup> in Iran (98.4%) although the specificity in those researches was different from this research (63.8 and 69.1%). The difference in specificity was due to differences in population characteristics of  $\beta$  thalassemia trait. The formula of Sirdah index was  $MCV - \text{erythrocyte count} - (3 \times Hb)$ . In the research conducted by Matos<sup>15</sup>, the mean MCV was 63.7 fL, erythrocyte count 5.47 (in  $10^6/\mu\text{L}$ ) and hemoglobin 11.5g/dL, so the mean value of the index was 23.73. In this research, on the other hand, the mean MCV was 61.87 fL, erythrocyte count 5.24 (in  $10^6/\mu\text{L}$ ) and hemoglobin 10.23 g/dL, so the mean value of the index was 25.94. This indicated that a small MCV could generate a small index value, however, the erythrocyte count and hemoglobin in this research were small, as a result, so the mean value of the index was greater than in Matos.<sup>15</sup> This means that  $\beta$  thalassemia trait subjects in this research tended to be more likely to have a value of  $>27$  compared to those in Matos' research resulting in a decreased specificity.

Optimal *cut-off* value of an index could be obtained by calculating the highest sensitivity and specificity that could be obtained by a *cut-off* value. In Mentzer index, the *cut-off* value selected was 13.44 resulting in a sensitivity of 78.6% and a specificity of 73.8%. In the RDW index, the *cut-off* value chosen was 233.4 resulting in a sensitivity of 87.8% and a specificity of 81.2%. In the Green-King index, *cut-off* value selected was 75.06 resulting in a sensitivity of 93.9% and a specificity of 83.8%. In the Sirdah index, selected *cut-off* value was 32.52 resulting in a sensitivity of 81.6% and a specificity of 85.0%. Figure 1 showed ROC curves formed for all indices. Based on the *area under the curve*, it was known that the most comprehensive index was Green-King (brown curve) in the first place, followed by RDW (green curve) in the second place,

Sirdah (purple curve) in the third place and Mentzer (blue curve) in the fourth place.

The determination of the optimal *cut-off* values was aimed to obtain the highest diagnostic value that could be generated by a test tool. In ROC curve in Figure 1, the optimal *cut-off* value was closest to the upper left corner of the ROC curve. The optimal *cut-off* value could also be determined from a table of SPSS showing optimum results of the sum of sensitivity and specificity. The new *cut-off* values obtained in this research were 13.44 for Mentzer index, 233.4 for RDW, 75.06 for Green-King, and 32.52 for Sirdah. This indicated that all of the new *cut-off* values were higher than the standard *cut-off* values that had already been set. The different optimal *cut-off* values among the researchers were because of differences in population of each region. In this research, both diseases were considered to have the same interest power, thus, the optimal *cut-off* values were used. *Cut-off* values can practically be modified according to clinical purposes. If it is wanted to obtain a higher sensitivity for screening needed, the *cut-off* values could be decreased, followed by a decrease in the specificity. In this research, erythrocyte index with the new *cut-off* value with the best diagnostic value was derived from the Green-King index with a sensitivity of 94.8% and a specificity of 83.7%. Based on these results, the use of Green-King index was suggested with a *cut-off* value of 75.06 to distinguish iron deficiency anemia from  $\beta$  thalassemia trait. The diagnostic value with a new *cut-off* value could provide better results because it could show a higher accumulation of sensitivity and specificity than using the determined *cut-off* values. Therefore, it is advisable to set a new *cut-off* value



**Figure 1.** ROC curve of the four indexes.

complying with the population in their respective regions in order to obtain the most optimal diagnostic value.

## CONCLUSIONS AND SUGGESTION

In conclusion, indices which could be applied to the people of Indonesia were Green-King index, RDW, Sirdah and Mentzer. However, the index which provided the best diagnostic value was Green-King. Mentzer index diagnostic values showed a sensitivity of 83.6%, specificity of 66.2%, PPV of 75.2%, NPV of 76.8%, PLR of 2.4 and NLR of 0.2. RDW index diagnostic values showed a sensitivity of 91.8%, specificity of 75%, PPV of 81.8%, NPV of 88.2%, PLR of 3.6 and NLR of 0.1. Green-King index diagnostic values shows a sensitivity of 96.9%, specificity of 67.5%, PPV of 78.5%, NPV of 94.7%, PLR of 2.9 and NLR of 0.04. While, Sirdah index diagnostic values showed a sensitivity of 92.8%, specificity of 58.7%, PPV of 73.3%, NPV of 87.0%, PLR of 2.2 and NLR of 0.1. Thus, the new optimal *cut-off* values obtained were 13.44 for Mentzer, 233.4 for RDW, 75.06 for Green-King and 32.52 for Sirdah. The Green-King index with the *cut-off* value of 75.06 was considered as a simple screening tool in order to distinguish iron deficiency anemia form  $\beta$ -thalassemia trait using automated blood cell instrument with RDW-CV parameters. Therefore, it was suggested that diagnostic testing should be conducted in different populations of various areas.

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