Reliability of Different RBC Indices and Formulas in the Discrimination of β-Thalassemia Minor and Iron Deficiency Anemia in Surabaya, Indonesia

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Abstract

In this study, we evaluated the RBC indices in differentiating β-thalassemia minor and iron deficiency anemia in a healthcare center. This was an observational analytic study with a cross-sectional design using blood specimens of children aged 3 years to 17 years with microcytic hypochromic anemia based on the results of complete blood counts and evaluation of peripheral blood smears. Calculation of the RBC indices was performed as an initial screening to differentiate β-thalassemia minor and iron deficiency anemia. Iron profile examination and hemoglobin electrophoresis were performed to confirm the diagnosis of β-thalassemia minor and iron deficiency anemia. The results of the independent samples t-test showed significant differences in Hb, MCV, MCH, and MCHC between β-thalassemia minor and iron deficiency anemia ($p < 0.05$). The results of the Mann Whitney test showed a significant difference in the Mentzer Index to distinguish between β-thalassemia minor and iron deficiency anemia ($p < 0.05$). Also, the results of the independent samples t-test showed significant differences in the Green & King formula, Sirdah *et al.* formula, and the Maltos and Carvalho Index ($p < 0.05$). The Green and King formula has a diagnostic sensitivity of 78.6% and a specificity of 76.6% and an accuracy of 78.03%. Various formulas based on the results of complete blood count parameters have been developed to detect β-thalassemia minor in areas with a high prevalence of β-thalassemia with different sensitivity and specificity. In this study, it showed that the Green and King formula has a diagnostic sensitivity of 78.6% and a specificity of 76.6%, and an accuracy of 78.03%. Green and King's formula can be applied as an initial screening to differentiate β-thalassemia minor and iron deficiency anemia

Keywords: *RBC indices, β-thalassemia minor, iron deficiency anemia.*

Introduction

WHO study shows that as many as 800 million children and women have anemia, which the population with the highest prevalence of anemia and the lowest hemoglobin concentration being in the Southeast Asian, Eastern Mediterranean, and African regions. Approximately 50% of cases of anemia are considered to be due to iron deficiency, other causes are inherited or acquired disorders that affect hemoglobin synthesis, red blood cell production, or red blood cell survival.[1]

The results of Basic Health Research from the Ministry of Health of the Republic of Indonesia (2013) have shown that 21.7% of the age of population ≥1 year, 28.1% of toddlers aged 12-59 months, and 37.1% of pregnant women are the proportion of anemia in Indonesia.[2] Differential diagnosis of microcytic hypochromic anemia is very important to be considered because the interpretation of its peripheral blood smear

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can be found in iron deficiency anemia and β-thalassemia trait.[3,4] Iron deficiency and β-thalassemia minor are best differentiated using serum ferritin level, serum iron level, total iron-binding capacity, transferrin saturation, and Hb A_2 level, along with a complete blood count (CBC) and examination of a peripheral blood film.[3] Carriers of β-thalassemia are usually clinically asymptomatic. However, they have characteristics of the CBCs with mean corpuscular volume (MCV) less than 80 fL and mean corpuscular hemoglobin (MCH) less than 27 pg.[5]

The ability and experience of a doctor in discriminating between β-thalassemia minor and iron deficiency anemia (IDA) clinically is very important because there are still difficulties in distinguishing between these two diseases. There are differences in the prognosis and management of those diseases, so the exclusion of iron deficiency anemia could be achieved mathematically using the red blood cell (RBC) parameters.[4,5] Various formulas using a complete blood count have been developed to detect β-thalassemia carriers in areas where the prevalence of thalassemia major is high; however, specificity and sensitivity vary across regions. [4,6]

In an attempt to simplify the differential diagnosis between IDA and β-thalassemia minor, several indices using blood cell count parameters have been suggested.[4] According to many studies, the formula developed by Green and King {[(MCV^2) x RDW] / (Hb x 100)}, Sirdah *et al*. Formula (MCV – RBC – 3xHb), Matos and Carvalho Index (1.91 x RBC) + (0.44 x MCHC), and Mentzer Index (MCV/RBC) have a good performance.[4,5,7] Capillary zone electrophoresis (CE) was able to detect the most common variants but differed concerning measurement HbA. The CE was able to measure HbA_2 in the presence of HbE. This is important to diagnose thalassemia and hemoglobinopathies.[8] Few data are available about the evaluation of the diagnostic reliability of different RBC indices and formulas to differentiate of the β-thalassemia minor and iron deficiency anemia (IDA) in Surabaya, Indonesia. In population or mass-screening programs especially in developing countries, these hematology indices could be applied where resources are limited.[7] Therefore, this study aimed to evaluate the sensitivity and specificity of different RBC indices and formulas in the differentiation of the β-thalassemia minor and iron deficiency anemia.

Materials and Methods

Study Subjects and Sample Collection

This was an observational study with a cross-sectional approach performed in all blood specimens in patients aged 3 years until 17 years with hypochromic (MCH <27 pg) microcytic (MCV <80 fl) anemia based on the results of a complete blood count (CBC) and evaluation of peripheral blood smears. A total of 223 subjects (124 males, 99 females) were studied in Dr. Soetomo General Academic Hospital, Surabaya, Indonesia between January 2019 and August 2019. The hematological index calculation (Mentzer Index, Green and King Formula, Sirdah *et al.* Formula, and Maltos and Carvalho Index) was performed as initial screening in distinguishing iron deficiency anemia and β-thalassemia minor. Examination of iron profiles (serum iron, total iron-binding capacity, and serum ferritin), and hemoglobin electrophoresis was carried out subsequently as confirmation of the diagnosis of iron deficiency anemia and β-thalassemia minor.

Inclusion criteria for iron deficiency anemia (IDA) subjects were microcytic hypochromic anemia with serum iron level <50 µg/dl, TIBC level >390 µg/dl, transferrin saturation <15%, and serum ferritin level <15 µg/L.[9] In addition, inclusion criteria for the β-thalassemia minor (BTMi) subjects were children with microcytic hypochromic anemia, had a family history (parents or siblings) of β-thalassemia. Their CBCs show hemoglobin (Hb) less than 13.5 g/dl, MCV less than 80 fL, and MCH less than 27 pg. Subjects with severe sepsis were not included in this study and none of the subjects had a combined case of β-thalassemia minor and iron deficiency anemia. Then, β-thalassemia minor is confirmed by quantitation of HbA_2, in which HbA_2 >3.5% indicates β-thalassemia minor.[3] The following indices and formulas were calculated and compared:

Green and King formula ([(MCV^2) × RDW]/Hb × 100).[10]

Sirdah *et al*. Formula (MCV – RBC – 3 × Hb).[7]

Matos and Carvalho Index (1.91 × RBC) + (0.44 × MCHC).[4]

Mentzer Index (MCV/RBC).[11]

Sensitivity [true positives/(true positives + false negatives)], specificity [true negatives/(true negatives + false positives)], positive predictive value [true positives/(true positives + false positives)], negative predictive value [true negatives/(true negatives + false negatives)], and accuracy [(true positives + true negatives)/total subjects] were calculated for each index and formula at wide-range of cut-off values.

Sample Preparation, Hematological and Clinical Chemistry Evaluations

Peripheral blood (3 mL) was collected in tubes containing K3EDTA (ethylenediaminetetraacetic acid) with a final concentration of 1.5 mg/mL. Complete blood count was examined using an automated hematology analyzer (CELL-DYN Ruby, Abbott® and Sysmex® XN-1000) in the laboratory unit of clinical pathology, Dr. Soetomo General Academic Hospital.

Hemoglobin electrophoresis tests were performed using a capillary electrophoresis system (MINICAP Sebia®) which able to measure HbA_2 in the presence of HbE. The capillary electrophoresis system does not require daily calibration, but normal HbA and HbA_2 migration controls are analyzed through each capillary daily before running QC materials or patient samples to ensure proper charge and function of the capillaries.[12] Normal HbA_2 control (Sebia®, France) for QC materials were also run daily.

Another sample of the peripheral blood (2 mL) was collected with BD Vacutainer® SST™ blood collection tubes contain spray-coated silica to aid in clotting and a polymer gel for serum separation to evaluate serum iron, total iron-binding capacity, and serum ferritin values.

Statistical Analysis

Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy were calculated for each measurement of the formulas. Descriptive statistics such as the mean, the median, and the standard deviation (SD) were calculated for hematological parameters (RBC, hematocrit, RDW-CV) and also age variable. The differences between the two groups parameter (β-thalassemia minor and iron deficiency anemia) were compared using the Mann-Whitney U test, because of the distribution of these parameters were non-normal. The independent samples t-test was used to compare the differences for hematological parameters (Hb, MCV, MCH, and MCHC) between these groups. The normality of data was evaluated using the Kolmogorov-Smirnov test.

Results

The Hb value in the BTMi group was 8.53±1.62 compared to the IDA group was 10.96±2.13 (p <0.001). MCV values of 71.95±6.76 and MCHC 30.96±1.86 in the BTMi group were lower than in the IDA group (76.48±4.85 and 32.20±1.45 with p <0.001). RDW-CV in the BTMi group was 20.15±4.77, significantly higher than the IDA group (14.6±3.28 with p <0.001). Erythrocyte count (RBC) was higher in the IDA group of 4.46±8.86 than in the BTMi group (3.86±0.81 with p <0.001) (Table 1).

Table 1. Hematological parameters of the group of β-thalassemia minor and iron deficiency anemia.

Parameter	BTMi (n: 159)		IDA (n: 64)	
	Range	Mean±SD	Range	Mean±SD
Hb (g/dL)	4.49-14	8.53±1.62*	5.07-16.3	10.96±2.13*
Hct (%)	13.6-43.8*	27.57±5.06	14.7-51.6*	34.15±6.89
RBC (x10^6/µL)	1.9-6.77*	3.86±0.81	2.06-6.05*	4.46±8.86
MCV (fL)	55.0-99.3	71.95±6.76*	63.4-90.7	76.48±4.85*
MCH (pg)	16.6-31.7	22.34±3.02*	20.0-28.7	24.59±2.11*
MCHC	26.5-34.9	30.96±1.86*	27.6-34.9	32.20±1.45*
RDW-CV (%)	8.3-34*	20.15±4.77	10.5-30.9*	14.6±3.28

Note: Hb: hemoglobin; RBC: red blood cell; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; RDW-CV: red cell distribution width-coefficient of variation.

*Significant, $p < 0.001$

Table 2. Evaluation of different RBC indices in for distinguishing in β-thalassemia minor and iron deficiency anemia.

RBC Indices or Formula	Sensitivity	Specificity	PPV	NPV	Accuracy
Mentzer Index	66%	62.5%	81.4%	42.6%	65.02%
Green and King Formula	78.6%	76.7%	89.3%	59%	78.03%
Sirdah *et al.* Formula	64.2%	64.1%	81.6%	41.8%	64.13%
Martos and Carvalho Index	27.7%	25%	47.8%	12.2%	26.9%

The data from the present study (Table 2) showed the Green and King formula has the highest sensitivity (78.6%) and specificity (76.6%) in the differentiation of BTMi and IDA. The highest PPV value was found in the Green and King formula (89.3%) and the lowest was in the Martos and Carvalho index (47.8%). The highest NPV value was found in the Green and King formula (59%) and the lowest was in the Martos and Carvalho index (12.2%). There were none of the differentiation indices with high sensitivity and specificity (100%) in differentiating BTMi and IDA, but the overall index was significant in differentiating BTMi and IDA ($p < 0.05$) against the gold standard examination of hemoglobin electrophoresis (HbA2).

Discussion

The ability and experience of a doctor in distinguishing between BTMi and IDA is very important because it has clinical implications where the two diseases have different aspects of etiology, prognosis, and management.[13] The most common causes of anemia are iron deficiency anemia (IDA) and thalassemia trait (TT).[1] In infants, the incidence of IDA is estimated to be between 20-25%. The results of the Basic Health Research of the Ministry of Health of the Republic of Indonesia (2013) showed that the proportion of anemia in toddlers aged 12-59 months is 28.1%.[2,14] Many investigators still have used hematological index calculations based on complete blood parameters as the initial screening to differentiate BTMi and IDA.

A capillary electrophoresis system for the examination of hemoglobin electrophoresis costs more than a routine CBC, and if we can routinely include an automatic alert signal in any routine CBC analyzed by the hematology analyzer, the referring physicians can get an indication of whether they need to perform further studies.[15]

The data from the present study (Table 1) showed significant differences in hematologic parameters between the BTMi and IDA groups. The higher RBC increase in the IDA group compared to the BTMi group was probably related to the administration of iron therapy in children with IDA. Vehapoglu *et al.* (2014) observed that the RBC increased in patients with IDA at the time of initiation of iron therapy and a decreased of the RBC at the end of iron therapy.[13] This is consistent with the study of Aslan and Altay (2003) that 36 out of 140 (26%) children aged 6 months to 48 months with nutritional IDA without bleeding, experienced a significant increase in RBC accompanied by an increase in Hb and Hct, as well as a decrease in MCV values. A continuous increased in RBC occurred in patients with higher RBC (high-RBC patients) up to 4 weeks of iron therapy compared with those with lower RBC (low-RBC patients).[16]

Evaluation of the hematological indices in this study showed the Green and King formula had the highest sensitivity (78.6%) and specificity (76.6%) in differentiating BTMi and IDA, followed by the Mentzer index (66% and 62.5%), the Sirdah *et al.* formula (64.2% and 64.1%), and the Martos and Carvalho index (27.7% and 25%). These results were in accordance with the study of Urrechaga *et al.* (2011) who showed that the Green and King formula had high sensitivity (91%), specificity (99.1%), Youden index (90.1%), and area under the curve (AUC) in differentiating BTMi and IDA.[17]

This study had several limitations such as no mutational analysis data was carried out in the study. The platelet parameters were not included in this study which are available in the CBCs result that may be helpful as discriminating guide for BTMi and IDA.

Conclusion

In summary, it was concluded that none of the differentiation indices or formulas provided 100% sensitivity and specificity for the discrimination of BTMi and IDA. For the population of children in Surabaya, Indonesia, applying the Green and King formula might be useful in the early or initial screening stages of children with the hypochromic microcytic anemia.

Conflict of Interest: The authors declare that they have no conflict of interest.

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References

1. WHO. The global prevalence of anaemia in 2011. Who [Internet]. 2011; 1–48. Available from: https://apps.who.int?iris/hendle/10665/177094.
2. Kemenkes RI. Riset Kesehatan Dasar RI 2013. Riset Kesehatan Dasar 2013. 2013. 1–306. Available from: https://www.depkes.go.id/resources/download/general/Hasil Riskesdas 2013.pdf
3. Keohane EM, Otto CN, Walenga JM. Rodak's Hematology: Clinical Principles and Applications. Sixth Edit. Keohane EM, Otto CN, Walenga JM, editors. St. Louis, Missouri: Elsevier; 424–442; 2020.
4. Matos JF, Dusse LMS, Borges KBG, de Castro RLV, Coura-Vital W, Carvalho M das G. A new index to discriminate between iron deficiency anemia and thalassemia trait. Rev Bras Hematol Hemoter. 2016; 38(3): 214–19.
5. Bordbar E, Taghipour M, Zucconi BE. Reliability of different rbc indices and formulas in discriminating between β-thalassemia minor and other microcytic hypochromic cases. Mediterr J Hematol Infect Dis. 2015; 7(1): 1–7.
6. Maskoen AM, Reniarti L, Sahiratmadja E, Sisca J, Effendi SH. Shine & Lal index as a predictor for early detection of β-thalassemia carriers in a limited resource area in Bandung, Indonesia. BMC Med Genet. 2019; 20(1): 1–6.
7. Sirdah M, Tarazi I, Al Najjar E, Al Haddad R. Evaluation of the diagnostic reliability of different RBC indices and formulas in the differentiation of the β-thalassaemia minor from iron deficiency in Palestinian population. Int J Lab Hematol. 2008; 30(4): 324–30.
8. Keren DF, Hedstrom D, Gulbranson R, Ou CN, Bak R. Comparison of Sebia Capillarys capillary electrophoresis with the primus high-pressure liquid chromatography in the evaluation of hemoglobinopathies. Am J Clin Pathol. 2008; 130(5): 824–31.
9. Hendarto A, Febriyanto R, Kaban RK. Defisiensi besi dan anemia defisiensi besi pada anak remaja obes. Sari Pediatr. 2018; 20(1).
10. Green RK. A new red cell discriminant incorporating volume dispersion for differentiating iron deficiency anemia from thalassemia minor.

Blood Cells. 1989; 15(3): 481–91.

11. Mentzer J. Differentiation of iron deficiency from thalassaemia trait. Lancet. 1973; 1(7808): 1973.

12. Greene DN, Pyle AL, Chang JS, Hoke C, Lorey T. Comparison of Sebia Capillarys Flex capillary electrophoresis with the BioRad Variant II high pressure liquid chromatography in the evaluation of hemoglobinopathies. Clin Chim Acta. 2012; 413(15–16): 1232–8.

13. Vehapoglu A, Ozgurhan G, Demir AD, Uzuner S, Nursoy MA, Turkmen S, et al. Hematological indices for differential diagnosis of beta thalassemia trait and iron deficiency anemia. Anemia. 2014; 2014(1): 1–8.

14. Widiaskara I, Pramitha P, Suryawan IWB, Ugrasena I. Gambaran hematologi anemia defisiensi besi pada anak. Sari Pediatr. 2016; 13(5): 362.

15. Roth IL, Lachover B, Koren G, Levin C, Zalman L, Koren A. Detection of β-thalassemia carriers by red cell parameters obtained from automatic counters using mathematical formulas. Mediterr J Hematol Infect Dis. 2018; 10(1): e2018008.

16. Aslan D, Altay C. Incidence of high erythrocyte count in infants and young children with iron deficiency anemia: Re-evaluation of an old parameter. J Pediatr Hematol Oncol. 2003; 25(4): 303–6.

17. Urrechaga E, Borque L, Escanero JF. The role of automated measurement of RBC subpopulations in differential diagnosis of microcytic anemia and β-thalassemia screening. Am J Clin Pathol. 2011; 135(3): 374–9.