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Concordance Test of Various Erythrocyte Indices for Screening of Beta Thalassemia Carrier

Reni Nurazizah[1], Renaldi Satria Handika[1], Edhyana Sahiratmadja[2], Yoyos Dias Ismiarto[3], Delita Prihatni[4]

[1] Faculty of Medicine, Universitas Padjadjaran, Bandung, Indonesia
[2] Division of Biochemistry and Molecular Biology, Department of Biomedical Sciences, Faculty of Medicine, Universitas Padjadjaran, Bandung, Indonesia. E-mail: e.sahiratmadja@unpad.ac.id
[3] Department of Orthopaedics and Traumatology, Faculty of Medicine, Universitas Padjadjaran/Dr. Hasan Sadikin General Hospital, Bandung, Indonesia
[4] Department of Clinical Pathology, Faculty of Medicine, Universitas Padjadjaran/Dr. Hasan Sadikin General Hospital, Bandung, Indonesia

ABSTRACT

Carrier screening for early detection of thalassemia in the general population needs a careful approach, especially in areas with limited health services. Various erythrocyte indices have been introduced as options for the first stage; however, the low-reliability value of these erythrocyte indices remains the problem. This study aimed to determine the most reliable index for screening beta-thalassemia carriers and distinguish it from iron deficiency anemia. A cross-sectional study was designed to explore thalassemia carrier status among medical students. Inter-rater reliability value of various indices was compared to Shine and Lal index. The Cohen's Kappa coefficient was calculated using SPSS v. 25.0. Among 320 respondents, 295 subjects were non-anemic and 25 were anemic subjects, whereas 105 subjects had low MCV and/or MCH values. Cohen's Kappa value showed moderate reliability results compared to Shine and Lal index for example Mentzer index (0.58), Ehsani index (0.57), Srivastava index (0.53), and Bordbar index (0.41), but showed very low-reliability results with Green and King index (0.04). New cut-off indices based on Kumar *et al.* were also compared, resulting in moderate reliability results. Since there was no Hb-electrophoresis test, the sensitivities and specificities of those indices could not be calculated. For this reason, a complete blood count can only be used for the early stages of screening for beta-thalassemia carriers, whereas Hb-electrophoresis and DNA tests were considered necessary to perform to confirm a diagnosis.

Keywords: Beta-thalassemia carrier, erythrocyte indices, iron deficiency anemia, Shine and Lal index, thalassemia screening

INTRODUCTION

Thalassemia is an inherited erythrocyte disorder caused by a failure to form globin protein in erythrocyte formation.[1] Indonesia is one of the countries located in the thalassemia belt, with a high mutation frequency in thalassemia genes.[2] Thalassemia carrier cases account for 3 to 10% of the entire population in Indonesia.[3] Screening is needed to prevent new cases of thalassemia major.[4] Screening can be carried out during the premarital, preconception, or prenatal period.[5] Definitive diagnosis of thalassemia uses hemoglobin analysis and DNA examination; however, erythrocyte indices such as MCV and MCH are generally used for screening due to their quick procedure and cost effectivity.[6] MCV and/or MCH values are parameters for early screening of thalassemia carrier according to the Ministry of Health in Indonesia.[2,7]

A blood smear of beta-thalassemia carrier has microcytic hypochromic erythrocytes, leading to low MCV and MCH; however, the same features are also found in Iron Deficiency Anemia (IDA).[6] Approximately 30% of the global population suffers from iron IDA.[8] The prevalence of anemia at the age of 15-24 years reaches 32% in Indonesia, with IDA being the most common anemia.[9,10] The definitive diagnosis to differentiate beta-thalassemia carrier and IDA is based on iron study and hemoglobin electrophoresis.[11] However, all of the tests are expensive, time-consuming, and not widely available in every health service in Indonesia.[12] Various formulas using a combination of erythrocyte indices have been used as screening for thalassemia and to distinguish it from iron deficiency anemia to prevent unnecessary iron therapy for beta-thalassemia carrier.[5,11,13]

Shine and Lal index is the index with the highest sensitivity reaching more than 98% in detecting beta-thalassemia carrier whereas Mentzer, Green and King, and Srivastava index have lower sensitivity.[14] Shine and Lal index could confirm all beta-thalassemia carrier and could be used for early screening.[15] Other study has shown that the Mentzer index has a higher reliability value than other indices.[16] Ehsani, Green and King, Srivastava, and Bordbar index are also the most frequently used index for screening.[5] Interestingly, Kumar has suggested that new cut-off values for all indices were better than the conventional indices.[17]

There are varying results of reliability values in populations. This study aimed to determine the prevalence of suspect beta-thalassemia carrier and the most reliable index for screening of beta-thalassemia carriers and to distinguish it from iron deficiency anemia in remote areas where laboratory facilities for Hb electrophoresis and/or DNA test are limited.

METHODS

A cross-sectional descriptive study was designed to assess the concordance between indices for screening beta-thalassemia carriers and to differentiate from iron deficiency anemia. Cut-off of various erythrocyte indices for differentiating beta-thalassemia carrier and iron deficiency anemia was presented in Table 1. Secondary data from a previous study were used, comprising complete blood count results of 320 medical students with ages ranging from 16 to 24 years.[18]

In brief, data were divided into subjects with anemia and non-anemia, according to WHO standards for Hb value based on gender (female <12 g/dL and male <13 g/dL). Data were then categorized into low MCV and/or MCH values and normal MCV and MCH values based on the flowchart of thalassemia screening by the Ministry of Health (Figure 1).[18] Mentzer index, New Mentzer, New Shine and Lal, Srivastava, New Srivastava, Green and King , Ehsani and Bordbar index were compared to Shine and Lal index as references in our population as previously described, along with calculation of their Cohen's Kappa coefficient to determine the inter-rater reliability value.[15]

Cohen's Kappa coefficient of 0-0.2 indicated a very low-reliability value, coefficient ≥ 0.2-0.4 indicated a low-reliability value, coefficient ≥ 0.4-0.6 indicated a moderate reliability value, coefficient ≥ 0.6-0.8 indicated strong reliability value, and coefficient ≥ 0.8-1 indicated very strong reliability value. The study was approved by the Ethical Committee of the Medical Faculty, Universitas Padjadjaran, Bandung, Indonesia, with the number 85/UN6.KEP/EC/2020. Data were processed using the IBM SPSS version 25.0 program.

Table 1. Cut-off of various erythrocyte indices for differentiating beta-thalassemia carrier and iron deficiency anemia

Erythrocyte Index		Formula	Cut-off	
			BTT	IDA
MCV			<80	≥80#
	New*		F: <71.8	F: ≥71.8#
			M: <73.4	M: ≥73.4#
MCH			<27	≥27#
	New*		F: <22.6	F: ≥22.6#
			M: <24.2	M: ≥24.2#
Mentzer		MCV/RBC	<13	≥13
	New*		<14.15	>14.15
Shine and Lal		MCV x MCV x MCH/100	<1530	≥1530
	New*		<891.1	>891.1
Srivastava		MCH/RBC	<3.8	≥3.8
	New*		<4.72	>4.72
Ehsani		MCV - (10xRBC)	<15	≥15
Bordbar		(80-MCV) x (27-MCH)	≥44.76	< 44.76
Green and King		MCV x MCV x RDW/Hb x 100	<65	≥65

Note: *New proposed by cut-off,[17,19]

#Normal individual; BTT, β-Thalassemia Trait , IDA; Iron Deficiency Anemia; F, female; M, male

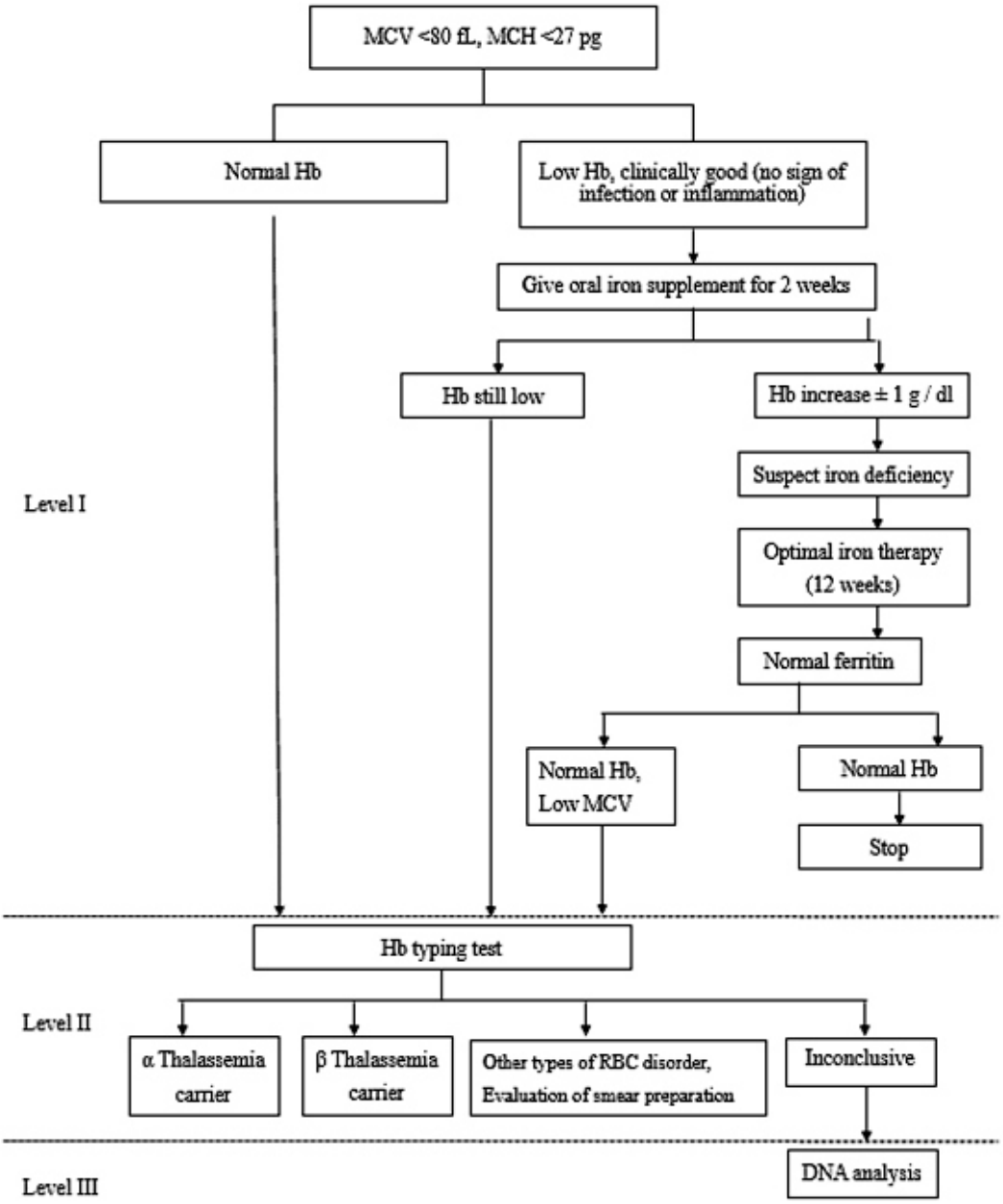

Figure 1. Flowchat of screening for thalassemia carrier

RESULTS AND DISCUSSIONS

A total of 320 data from medical students were used in this study, consisting of 295 (92.2%) normal Hb and 25 (7.8%) low Hb (anemia) results; 24 (96%) female and 1 (4%) male subject, with a median age of 19 years old (range 16-24 years). The distribution of individuals suspected of beta-thalassemia carrier or iron deficiency anemia according to various erythrocyte indices were classified by their anemia status characterized by low hemoglobin (Table 2). In addition, the distribution of individuals suspected of beta-thalassemia carrier or iron deficiency anemia was presented based on MCV and MCH values for microcytic and hypochromic anemia (Table 3).

The inter-rater reliability values between various erythrocyte indices compared to Shine and Lal index were presented in Table 4.

Table 2. The distribution of suspected beta-thalassemia carrier or iron deficiency anemia according to various erythrocyte indices based on hemoglobin value among medical students in Bandung

		Hemoglobin	
		Low N (%)	Normal N (%)
MCV and/or MCH	Low	22 (88) [a]	83 (28.1) [c]
	Normal	3 (12) [b]	212 (71.9)
New MCV and/or MCH	Low	11 (44) [a]	4 (1.4) [c]
	Normal	14 (56) [b]	291 (98.6)
Mentzer index	<13	8 (32) [a]	7 (2.4) [c]
	≥13	17 (68) [b]	288 (97.6)
New Mentzer index	< 14.15	10 (40) [a]	27 (9.2) [c]
	≥14.15	15 (60) [b]	268 (90.8)
Shine and Lal index	<1530	15 (60) [a]	16 (5.4) [c]
	≥1530	10 (40) [b]	279 (94.6)
New Shine and Lal index	<891.1	8 (32) [a]	1 (0.3) [c]
	>891.1	17 (68) [b]	294 (99.7)
Srivastava index	<3.8	8 (32) [a]	4 (1.4) [c]
	≥3.8	17 (68) [b]	291 (98.6)
New Srivastava index	<4.72	12 (48) [a]	37 (12.5) [c]
	>4.72	13 (52) [b]	258 (87.5)
Green and King index	<65	5 (20) [a]	121 (41) [c]
	≥65	20 (80) [b]	174 (59)
Ehsani index	<15	7 (28) [a]	6 (2) [c]
	≥15	18 (72) [b]	289 (98)
Bordbar index	≥44.76	10 (40) [a]	12 (4.1) [c]
	<44.76	15 (60) [b]	283 (95.9)

Note. [a] suspect BTT, [b] suspect IDA, [c] suspected of another hemoglobinopathy. BTT, β-thalassemia trait; IDA, Iron Deficiency Anemia

Thalassemia screening in college students is an important attempt to increase awareness about thalassemia in individuals of childbearing age and to prevent thalassemia major after marriage due to lack of information about the risk of thalassemia carrier as a partner.[3] Pre-marital screening has been carried out in various countries such as Cyprus. Screening at this stage has resulted in up to zero birth of new cases of thalassemia major per year in that country, suggesting that premarital is the most appropriate time to perform thalassemia screening.[5]

There is no legal prohibition against intermarriage between thalassemia carrier in Indonesia. However, providing proper education to couples with beta-thalassemia carrier about the consequences in the future after marriage is one preventive attempt. Medical students together with health workers should know and understand the inheritance of beta-thalassemia. A study shows the importance of continuing medical education in terms of prevention and education to increase public awareness of the early detection of beta-thalassemia.[18]

The similarity of characteristics of beta-thalassemia carrier and iron deficiency anemia sometimes makes it so difficult to differentiate one from the other. If a beta-thalassemia carrier is misdiagnosed as iron deficiency anemia, iron therapy is unnecessary because the Hb value will not improve. Moreover, if the thalassemia carrier individual is married to a thalassemia carrier, it can lead to the development of thalassemia major in their offspring.[12] Nowadays, a rapid and inexpensive calculation method based on the erythrocyte indices is often used for the early screening of beta-thalassemia. The sensitivity and specificity of erythrocyte indices have been proven by many studies.[11,17,20] Furthermore, there are variations in the mutation spectrum in thalassemia genes in different population.[21] Therefore, it is necessary to determine an erythrocyte index with a high diagnostic value in each population for mass screening purposes. There were students with low MCV and/or MCH values (n105;32.8%) who were not anemic. The highest percentage of subjects with normal Hb but suspected of hemoglobinopathy was obtained by the Green and King index (Table 2). A study by Maskoen *et al.* showed that Shine and Lal index was a good parameter and could be used for screening beta-thalassemia carrier in the general population.[15]

Table 3. The distribution of suspected beta-thalassemia carrier or iron deficiency anemia according to various erythrocyte indices based on MCV (microcytic) and MCH (hypochromic) values among medical students in Bandung

		MCV and/or MCH Value	
		Microcytic/Hypochromic N (%)	Normal N (%)
Mentzer Index	<13	15 (14.3)	0 (0)
	≥13	90 (85.7)	215 (100)
New Mentzer index	<14.15	26 (24.8)	11 (5.1)
	>14.15	79 (75.2)	204 (94.9)
Shine and Lal index	<1530	31 (29.5)	0 (0)
	≥1530	74 (70.5)	215 (100)
New Shine and Lal index	<891.1	9 (8.6)	0 (0)
	>891.1	96 (91.4)	215 (100)
Srivastava index	<3.8	12 (11.4)	0 (0)
	≥3.8	93 (88.6)	215 (100)
New Srivastava index	<4.72	36 (34.3)	13 (6)
	>4.72	69 (65.7)	202 (94)
Green and King index	<65	39 (37.1)	87 (40.5)
	≥65	66 (62.9)	128 (59.5)
Ehsani index	<15	13 (12.4)	0 (0)
	≥15	92 (87.6)	215 (100)
Bordbar index	≥44.76	12 (11.4)	10 (4.7)
	<44.76	93 (88.6)	205 (95.3)

Table 4. Summary of inter-rater reliability values between various erythrocyte indices compared to Shine and Lal index

Erythrocyte Indices	Cohen's Kappa Value
Mentzer index	0.58
New Mentzer index	0.54
New Shine and Lal index	0.42
Green and King index	0.04
Ehsani index	0.57
Bordbar index	0.41
Srivastava index	0.53
New Srivastava index	0.58

The highest agreement value with Shine and Lal index has been shown by the Mentzer index and the New Srivastava index, whereas the Green and King index had the lowest agreement value (Table 4). However, a study shows that the Mentzer index was a reliable index with a sensitivity of 89%, specificity of 87.9%, and Youden's index of 76.9%.[22] In another study, the results of the Mentzer index in patients with a combination of iron deficiency anemia and thalassemia carriers were the same as for patients only with cases of thalassemia carriers, suggesting that the Mentzer index could differentiate carriers of non-thalassemic patients regardless of iron status.[23] This study showed the same results that Mentzer index had the highest agreement value with Shine and Lal index to determine beta-carrier thalassemia and to differentiate it with iron deficiency anemia.

A new cut-off of the Srivastava index could detect a higher beta-thalassemia carrier than the previous cut-off Srivastava index. It was confirmed by a study by Kumar, which showed that the new cut-off of the Srivastava index had higher sensitivity and specificity value than the previous cut-off of the Srivastava index, indicating that the new cut-off Srivastava index can be used for screening beta-thalassemia carrier.[17] Kappa value between the New Mentzer index and Shine and Lal index was 0.54, indicating that there was moderate reliability. Furthermore, the New Mentzer index had high sensitivity and specificity at 86.5% and 88.4%, respectively.[17]

Kappa value between Srivastava index and Shine and Lal index was 0.53, suggesting that there was moderate reliability similar to the results in a study by Harahap *et al*., which found a moderate and significant agreement between Srivastava index with the result of Hb electrophoresis.[16] Furthermore, the Green and King index had a low agreement value in differentiating beta-thalassemia carrier from iron deficiency anemia, indicating that there was a high number of false-positive subjects. However, if compared to suspect beta-thalassemia carrier based on MCV and

MCH values, the Green and King index had the highest index in detection of beta-thalassemia carrier in subjects with microcytic and/or hypochromic anemia, in contrast to the result by Plengsuree suggesting that Green and King index had higher Youden index value, resulting in high sensitivity and specificity value.[23]

No performance of Hb-electrophoresis was the only limitation in this study. This remains a challenge to detect early cases of thalassemia carriers in the limited resource area.

CONCLUSIONS AND SUGGESTIONS

Various erythrocyte indices have different concordances from one to another. For this reason, a complete blood count can only be used for early stages of screening for beta-thalassemia carriers.

Since various erythrocyte indices have different sensitivity and specificity across the population, these indices can only be considered as the first parameters in the limited resources area for screening beta-thalassemia carrier in the population to prevent high false-negative results, as well as for differentiating beta-thalassemia carrier and iron deficiency anemia. Further studies are needed to confirm the finding by using Hb electrophoresis and DNA analysis as the gold standard.

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