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Screening for beta-thalassemia trait; applicability of red cell indices and parameters – A study in Sri Lanka

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ISSN: 1658-3639

PUBLISHER: Qassim University

ABSTRACT

Objective: Red cell indices and parameters are used to screen beta-thalassemia trait (BTT). Different red cell indices and formulae used to discriminate BTT in different populations show inconsistent results.

Methods: A retrospective study was performed to assess reliability of 11 red cell indices, parameters and formulae in differentiating BTT from non-BTT in a cohort of individuals referred for confirmation of BTT.

Results: Of 111 individuals, 79 were females and 32 were males. Of the total, 89 were confirmed to have BTT by Hb A2 quantification. The mean age of the group was 29.9 ± 19.2 years. The mean Hb concentration, MCV and MCH in BTT group were 10.45 ± 1.6 g/dL, 62.1 ± 5.4 fl, and 19.7 ± 1.7 pg, respectively. The mean red cell count in BTT group was $5.3 \pm 0.8 \times 10^9/L$ while in non BTT group it was $4.7 \pm 0.7 \times 10^9/L$. The highest specificity (86.4%) was shown by Sirdah, Sriwastava and England and Fraser indices, but their sensitivities were 61.8%, 57.3%, and 32.6%. The lowest number of false positives ($n = 3$, 13.6%) was shown by Srivastava, Sirdah and England and Fraser indices. Shine and Lal index showed 100% sensitivity and NPV and 12 false positives. MCV and MCH showed results similar to Shine and Lal index with 16 false positives each.

Conclusion: Use of Shine and Lal index in screening programs of BTT is superior to all the other indices and formulae. To confirm the findings of this study, further studies are recommended to be carried out in Sri Lanka on different ethnicities.

Keywords: Beta-thalassemia carrier, beta-thalassemia trait, red cell indices, red cell parameters, screening

Introduction

Thalassemia is the most common hereditary hemolytic anemia in the world. It is an autosomal recessive disease characterized by varying degrees of anemia. The genetic defects affect the synthesis of proteins called globins which are components of hemoglobin; the oxygen transport pigment in the red cells. Impaired synthesis of globin chains lead to compromised hemoglobin synthesis thus anemia.^[1,2]

In an individual, there are two types of globin genes (alpha and beta). The products of these genes, alpha, and beta globin chains are paired to form adult hemoglobin (HbA). Hb A is the main hemoglobin present in blood (over 95%) in any healthy individual. The thalassemias are named based on the affected gene or the globin chain thus mainly are of two types; alpha and beta based on the affected genes alpha and beta, respectively. The presence of mutated beta-globin gene/s is called beta-thalassemia.^[1,2]

An individual harbor two beta-globin genes, each inherited from one parent. The presence of one defective beta gene (inherited from one parent) is beta-thalassemia trait (BTT) (thalassemia carrier state or thalassemia minor). It is a mild disease and in some instances it is not considered as a disease as the individuals with BTT can have normal life span and can have even near normal hemoglobin. Inheritance of mutated beta-globin genes from both parents lead to beta-thalassemia major. The marriages between two individuals with thalassemia trait carry 25% risk or chance of having a baby with thalassemia major. It manifests in infancy with severe anemia. Thalassemia major individuals need lifelong blood transfusions for survival. They have higher morbidity and mortality due to many reasons including iron overload due to regular blood transfusions, endocrinopathies, infections and poor growth, and pubertal problems with inadequate iron chelation. The stem cell transplant early in the life (by the age of 5 years for the best outcome) is the only method available for cure at present. Therefore, management of individuals

with thalassemia major consumes a significant proportion of health-care expenditures in any country.

The countries such as Iran, Cyprus, Greece, and Bangladesh with the high prevalence of thalassemia adopt comprehensive screening programs to identify individuals with thalassemia trait to counsel and to prevent thalassemia major births. Hence, countries having higher prevalence of thalassaemic diseases introduced various policies and procedures to screen and identify individuals with thalassemia trait.^[3-5]

The diagnosis of carrier state may be missed unless specific tests are performed as beta-thalassemia minor individuals are usually asymptomatic. Women with single mutated beta gene can have mild anemia which is worsening during pregnancy even requiring blood transfusions.^[6] In traditional practice, full blood count test related parameters, indices or formulae are used to screen thalassemia trait. Thalassaemia screening program in Sri Lanka uses mean corpuscular volume (MCV) of 80 fL and/or mean corpuscular hemoglobin (MCH) of 27 pg as the cutoff values to decide on confirmatory test.^[6] In countries, where nutritional deficiencies are common, the presence of coexisting iron deficiency, folate deficiency, and chronic disease can complicate interpretation of the red cell parameters and indices as it can share and alter these red cell parameters.^[6] The confirmation of BTT needs quantification of hemoglobin A2 either by high-performance liquid chromatography (HPLC) or by capillary electrophoresis (CE). Both these tests are costly and not widely available in many countries. Performance of confirmatory tests in all the suspected individuals without a strong justification increases the laboratory workload unacceptably, number of unwanted tests, and health-care budget. In addition, it can lead to an unwanted frustration in non-BTT individuals in some social settings due to social stigma of being diagnosed as having a blood disorder.

Applicability of different red cell parameters and red cells indices in determination of potential beta-thalassemia carrier status and their value in predicting BTT had been assessed in many studies.^[7-13] The indices that have been evaluated and in use include red cell count, Shine and Lal, Mentzer, Srivastava, Ehsani, Sirdah, red cell distribution width index (RDWI), Green and King, England and Fraser, mean density of Hb/L of blood index (MDHL), and mean cell Hb density index (MCHD).^[7]

Usefulness, ability or strength of these different indices and parameters to discriminate iron deficiency or non-BTT individuals from BTT in different populations show inconsistent results in many studies.^[7-9] According to a study carried out by Kumar *et al.*, the Mentzer index appears to be the most efficient index in discriminating BTT from iron deficiency. However, they recommend Shine and Lal as the best screening tool.^[14] Zaghoul *et al.* in their study have shown that England and Fraser index is the best for both men and women.^[15] With 100%

specificity and positive predictive value, RDW/RBC formula was considered the best in the study carried out by Plengsuree *et al.*^[10] Therefore, the use of these parameters in routine practice to identify BTT individuals while discriminating them from non-BTT individuals who mimic clinical manifestations and basic laboratory results of BTT is challenging. Since, there are evidences to suggest the effectiveness of use of individual indices and parameters in predicting BTT in some studies,^[9,10] it is important to study the usefulness and power of combined indices as screening tools as well. By a successful screening test it is expected to minimize false positives as much as possible thus minimizing the unnecessary costs on confirmatory tests and the psychological burden.

This retrospective study was carried out in a private sector hematology laboratory using all the referrals received for the confirmation or exclusion of BTT within a period of 1 year. The objective of the study was to assess the efficacy of eleven known indices and formulae in predicting BTT which will not miss true positives and has minimum false positives to reduce the unwanted confirmatory tests.

Materials and Methods

Ethical clearance for the study was obtained from the ethical review committee, Faculty of Medicine, University of Ruhuna and the institution's approval was obtained to access the database to carry out the project. No personal identification data were collected. There were no sample collections or involvement of patients in the study as this was a retrospective study carried out using the available data base.

All the test requests received for the quantification and identification of hemoglobins to diagnose thalassaemic conditions within a period of 1 year was the study population. The patients without automated full blood count (FBC) (some countries use the abbreviation CBC or CBE) results were excluded from the study. From the documented data of each patient, red blood cell parameters of the FBC reports {red blood cell count (RBC count), hemoglobin (Hb), packed cell volume (PCV), MCV, MCH, and red cell distribution width (RDW) – performed using Sysmex fully automated analyzer} and hemoglobin chromatography findings (Hb HPLC – performed by using Bio-Rad HPLC machine) were retrieved from the laboratory database.

All these tests had been performed maintaining the highest standards while integrating a comprehensive and regular internal and external quality control process to assure reliability. The laboratory is accredited as per ISO 15189 standard.

Based on HPLC results, the diagnosis of BTT was made when Hb A2 quantity of the study subjects was between 3.5% and 7.5% as per the accepted norm. Based on HPLC confirmation, the study population was divided into two; individuals with

confirmed BTT (with HbA2 values between 3.5% and 7.5%) and non-BTT (with HbA2 <3.5%).

Using the predefined cutoff values of 11 indices (Shine and Lal, Mentzer, Shrivastava, Eshani, Sirdah, RBC distribution width index, Green and King, England and Fraser, Ricera, MDHL index, and MCHD index), the subjects in each group (BTT and non-BTT) were classified again in to BTT and non-BTT and the sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV), and Youden's index of each index were calculated [Table 1].

Results

During the study period, there were total of 111 requests received by the laboratory for the diagnosis of BTT. Of those, 79 were females and 32 were males. The age of the study subjects ranged from 1 to 74 years and the mean age of the group was 29.9 ± 19.2 years. Of the study subjects, 89 had Hb A2 within 3.5%–7.5% confirming the diagnosis of BTT (HbA2) while 22 had normal chromatography (HbA2 <3.5%) excluding the diagnosis of it. The Hb concentration in BTT group ranged from 6.5 g/dL to 14.4 g/dL with a mean of 10.45 ± 1.6 g/dL. The mean values of MCV and MCH in BTT group

were 62.1 ± 5.4 fl and 19.7 ± 1.7 pg, respectively [Table 2]. The mean red cell count (RBC) in BTT confirmed group was $5.3 \pm 0.8 \times 10^9/L$ while in non-BTT group it was $4.7 \pm 0.7 \times 10^9/L$ ($P < 0.05$).

The sensitivity and specificity of red blood cell count (RBC) alone in diagnosing BTT were 62.9% and 66.7%, respectively. Shine and Lal index showed the highest sensitivity (100%) and the highest negative predictive value (100%) but its specificity was low (45.5%) and had 12 (54%) false positives. The highest specificity (86.4%) was shown by Sirdah, Sriwastava and England and Fraser indices, but their sensitivities were 61.8%, 57.3%, and 32.6% and the negative predictive values were 35.8%, 30.6%, and 24.0%, respectively. The lowest number of false positives ($n = 3$, 13.6%) was shown by Srivastava, Sirdah and England and Fraser indices [Table 3].

Discussion

There are remarkable inconsistencies among the results obtained in different studies carried out using different red cell indices.^[7-9,13,16-20] Findings of this study provide some interesting directives. Four individuals (4.5%) with confirmed thalassemia trait had Hb concentrations of 14 g/dL or above.

Table 1: Different RBC indices, mathematical formulae and cutoff values used to differentiate BTT from non-BTT^[8]

Index	Formula	BTT (cutoff)	Non-BTT (cutoff)
Shine and Lal	$MCV \times MCV \times MCH/100$	<1530	>1530
Mentzer	MCV/RBC count	<13	>13
Srivastava	MCH/RBC count	<3.8	<3.8
Ehsani	$MCV - 10 \times RBC$	<15	>15
Sirdah	$MCV - RBC - 3 \times Hb$	<27	>27
RDWI	$MCV \times RDW/RBC$	<220	>220
Green & King	$MCV \times MCV / (Hb \times 100)$	<72	>72
England & Fraser	$MCV - (5 \times Hb) - RBC$ count	<0 (negative)	>0 (positive)
Ricerca	RDW/RBC count	<3.3	>3.3
MDHL	$(MCH/MCV) \times RBC$ count	>1.63	<1.63
MCHD	MCH/MCV	>0.3045	<0.3045

BTT: Beta-thalassemia trait, RDWI: Red cell distribution width index, MDHL index: Mean density of Hb/liter of blood, MCHD index: Mean cell Hb density, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, RBC: Red blood cell count, RDW: Red cell distribution width

Table 2: Full blood count parameters of the study group

		Hb (g/dl) (mean±SD)	MCV (fl) (mean±SD)	MCH (pg) (mean ± SD)
Non-BTT (n=22)	Male (n=06)	12.1±2.2	75.05±10.2	24.7±3.7
		>12, n=01	>80, n=01	>27, n=01
		<12, n=05	<80, n=05	<27, n=05
	Female (n=16)	11.1±2.0	76.71±10.3	24.2±3.9
		>12, n=05	>80, n=05	>27, n=05
		<12, n=11	<80, n=11	<27, n=11
BTT (n=89)	Male (n=26)	11.8±1.7	60.4±5.7	19.7±1.9
		>12, n=11	>80, n=0	>27, n=0
		<12, n=15	<80, n=26	<27, n=26
	Female (n=63)	9.8±1.1	62.8±5.1	19.7±1.6
		>12, n=02	>12, n=0	>12, n=0
		<12, n=61	<12, n=63	<12, n=63

BTT: Beta-thalassemia trait, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin

Table 3: Sensitivity, specificity, negative predictive value, positive predictive value, and Youden's index of each discrimination index

Indices (cutoffs)	BTT (n: 89)	Non-BTT (n: 22)	Correctly diagnosed (%)	Sensitivity	Specificity	NPV	PPV	Youden's index
Mentzer								
<13 BTT	60	4	70.2	67.4	81.8	38.3	93.7	0.49
>13 non-BTT	29	18						
RBC count (10 ⁶ /L)								
>5 BTT	56	7	63	62.9	66.6	29.8	88.8	0.29
<5 non-BTT	33	14						
RDWI								
<220 BTT	62	6	70.2	69.6	72.7	37.2	91.2	0.42
>220 non-BTT	27	16						
Shine and Lal								
<1530 BTT	89	12	89.1	100	45.5	100	88.1	0.45
>1530 non-BTT	0	10						
Srivastava								
<3.8 BTT	46	3	58.5	51.7	86.4	30.6	93.9	0.38
>3.8 non-BTT	43	19						
Green and King								
<65 BTT	55	6	63.9	61.8	72.7	32.0	90.2	0.34
>65 non-BTT	34	16						
Sirdah								
<27 BTT	55	3	66.6	61.8	86.4	35.8	94.8	0.48
>27 non-BTT	34	19						
Eshani								
<15 BTT	60	4	70.3	67.4	81.8	38.3	93.8	0.49
>15 non-BTT	29	18						
England and Fraser								
<0 BTT	29	3	43.2	32.6	86.4	24.0	90.6	0.19
>0 non-BTT	60	19						
Ricerca								
<4.4 BTT	82	20	75.7	92.1	9.0	22.2	80.4	0.012
>4.4 non-BTT	7	2						
MDHL								
>1.63 BTT	52	7	60.4	58.4	68.1	28.8	88.1	0.26
<1.63 non-BTT	37	15						

BTT: Beta-thalassemia trait, NPV: Negative predictive value, PPV: Positive predictive value, RDWI: Red cell distribution width Index, MDHL index: Mean density of Hb/liter of blood

Accordingly, use of Hb concentration to discriminate BTT is inappropriate and misleading as it can dangerously exclude a significant proportion of individuals with BTT. As this was a retrospective study, nutritional status of the study subjects could not be assessed by means of dietetic history or by biochemical methods although it is known to influence the interpretation of red cell parameters.

In Sri Lanka, the National Thalassaemia screening program uses MCV below 80 fl and MCH below 27 pg as the cutoff values to decide on performing confirmatory test. The application of these cutoff values to our study sample showed 100% sensitivity and 27.2% specificity and assembled all true BTT individuals and additional 16 non-BTT individuals ($n = 22$) in to beta-thalassaemia suspected group. High red blood cell count has been used in differentiating BTT from non-BTT in some studies and was considered one of the most accurate indices available.^[9] In this study too RBC showed high sensitivity (94.4%) but low specificity (13.6%) with a Youden's index of 0.08. A study carried out by Vehapoglu *et al.* has shown high sensitivity (94.8%) but low specificity (50%) with RBC.^[9]

This shows that RBC alone is not a reliable tool to distinguish BTT from non BTT.

Ferrara *et al.* demonstrated high sensitivity (78.9%) of RDWI in a study carried out in children with mild microcytic anemia, while England and Fraser index had the highest specificity (99.1%) with the highest Youden's index (0.64).^[16] As per the study carried out by Eshani *et al.*, the best discrimination index was the Mentzer index (90.1%) according to Youden's criteria, followed by the Eshani *et al.* index (85.5%). In their study, Mentzer and Eshani indices correctly diagnosed 94.7% and 92.9% of the study subjects with and without BTT, respectively.^[17]

A good screening test to detect BTT should have high sensitivity and high specificity and less number of false positives. A screening test with high sensitivity will detect almost all the patients and the minimum number of false positives would minimize the unnecessary cost of confirmatory tests. Studies have been carried out to determine indices based better formulae.^[12,15,21]

In this study, the lowest number of false positives ($n = 3$, 13.6%) was shown by Srivastava, Sirdah and England and Fraser indices but their sensitivities and specificities were low. Shine and Lal index showed the highest sensitivity (100%) and the highest negative predictive value (100%). Its specificity was 45.5%, Youden's index was 0.45, positive predictive value was 88.1%, and percentage correctly diagnosed was 89.2%. It showed no false negatives but 12 (54%) false positives.

Similar to the findings of this study, Vehapoglu *et al.*,^[22] Bordbar *et al.*,^[23] Zahid *et al.*,^[24] Kumar *et al.*,^[14] Roth *et al.*,^[25] and Mustafa *et al.*^[26] showed Shine and Lal index to be the most sensitive. Of them only Vehagpolu *et al.*, Zahid *et al.*, and Mustafa *et al.* demonstrated 100% sensitivity for Shine and Lal index, as shown in this study.

Since, finding a screening test with 100% sensitivity and specificity is frequently not possible, we recommend, to use Shine and Lal index as a screening test since it detects all true positives.

We conclude that the reliability of the known formulae and indices other than Shine and Lal index in predicting BTT is less. Although, they decrease the costs of confirmatory tests by decreasing the number of false positives, they dangerously miss significant numbers of true BTT patients. Since, detection of all true positives is more important, we confirm that the use of Shine and Lal index in the screening programs of BTT is superior to all the other indices and formulae. To confirm the findings of this study, further studies are recommended to be carried out in Sri Lanka on different ethnicities.

Authors' Declaration Statements

Ethics approval and consent to participate

Ethics approval was obtained from the Ethical Review Committee of Faculty of Medicine, University of Ruhuna, Sri Lanka. The consent of participants was not a requirement since the data were obtained from existing database. Institutional permission and approval were obtained to retrieve data which were mandatory to obtain ethical clearance.

Availability of data and material

The data that support the findings of this study are available on request from the corresponding author, Wickramaratne KAC. The data are not publicly available since we have not obtained permission from ethics review committee for that.

Competing interests

The authors have no competing interests.

Funding statement

Authors received no funding from any institution or organization to carry out this study

Authors' Contributions

The first author designed the work, collected and interpreted data, verified the analytical method, and critically revised the final manuscript with important intellectual content.

The second author performed the computations and analysis, took the lead in writing the manuscript.

Both the authors discussed the results and contributed to the final manuscript.

Acknowledgment

We acknowledge the administrative team of Lanka Hospitals Sri Lanka, Head Laboratories Lanka Hospitals Diagnostics (LHD) and the technical team of the Department of Hematology, LHD.

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