

USEFULNESS OF RED CELL INDICES IN DIFFERENTIATING MICROCYTIC HYPOCHROMIC ANEMIAS

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ABSTRACT

Background: Iron Deficiency Anemia and beta-thalassemia trait are the two most common causes of microcytic hypochromic anemia. In this study we compared the validity of the various indices to differentiate between these two conditions.

Material & Methods: A total of 312 patients from 7th December 2007 to 4th April 2009 were studied retrospectively by calculating seven discrimination indices i.e. Mentzer, Shine & Lal, Srivastava, England & Fraser, Ricerca, Green & King and Red cell Distribution Width Index (RDWI). The study included 213 BTT cases (HbA2 > 3.5% and < 8.0%) and 89 non BTT cases (HbA2 < 3.5%). The number of correctly identified cases were determined and sensitivity, specificity, positive and negative predictive value and Youden index of each discrimination index were calculated.

Results: None of the discrimination index showed 100% sensitivity and specificity. The percentage correctly diagnosed is highest for RDWI (88.14%) followed by Mentzer index (86.85%). Youden index is also highest for RDWI (72) and Shine & Lal (72) followed by Mentzer (70).

Conclusion: RDWI, Mentzer and Shine & Lal are the most reliable formulae in discrimination between iron deficiency anemia and beta-thalassemia trait.

KEY WORDS: Red cell indices, Microcytic anemia, Beta-thalassemia trait, Iron deficiency.

INTRODUCTION

It is estimated that 1.5% of the world population is beta thalassemia carriers, about 60,000 new carriers are born each year and 50% of world thalassemia minor are in South-east Asia.¹ Beta thalassemia is the most common inherited disorder in Pakistan with an estimated carrier rate of 5%.² Effective population screening for thalassemia trait can dramatically decrease the incidence of thalassemia major births, for which peripheral smear examination is an invaluable tool³ along with red blood cell indices.⁴

Iron deficiency is the most common single nutrient disorder in humans in both developing and developed countries⁵ Both Iron Deficiency Anemia (IDA) and beta-thalassemia trait (BTT) are the most frequent causes of microcytic hypochromic anemia.^{6,7}

Carriers of beta thalassemia have been considered patients in whom iron deficiency is difficult to exist because dyserythropoiesis enhances enteric iron absorption. However, iron deficiency might coexist or develop in many carriers of beta thalassemia.^{8,9}

In the last four decades many formulae have been proposed to discriminate between the two common causes of microcytic hypochromic anemia.¹⁰⁻¹⁶ These formulae, based on Hb, RBC count, MCV and RDW, are useful in uncomplicated cases but not in patients who have both IDA and BTT, a situation not uncommon in Indian subcontinent.¹⁷

An ideal discrimination index should have a high sensitivity and specificity and be easy to calculate.

We compared the validity of various discrimination indices in differentiating BTT from IDA by calculating sensitivity, specificity, positive & negative predictive value and Youden's index. Youden's index takes into account both sensitivity and specificity and gives an appropriate measure of validity of particular technique.^{18, 19}

MATERIAL AND METHODS

All 312 patients who were referred to Thalassemia Control Project Peshawar, Pakistan for Haemoglobin A2 (HbA2) estimation from 7th December 2007 to 4th April 2009 were retrospectively analyzed.

The blood counts including the red blood cells (RBC) indices and Red cell distribution width (RDW) were performed on Hema Count Plus (Human Germany) fully automated working on the principal of impedance method. Hb electrophoresis for HbA2 estimation by alkaline

cellulose acetate electrophoresis machine model Beckman Coulter Model 6558 USA was used.

BTT (n=223) was diagnosed with HbA2 >3.5% and <8%²⁰ plus MCV <76 fl and/or MCH <27 pg. While the non BTT group (n=89) were those with HbA2 <3.5%.

Table 1: Red cell counter based formulae to differentiate BTT from IDA.

Index	Formula	BTT	IDA
England & Fraser ¹⁰	MCV-(5xHb)-RBC	<0 (neg)	>0 (pos)
Mentzer ¹¹	MCV/RBC count	<13.0	>13.0
Srivastava ¹²	MCH/RBC count	<3.8	>3.8
Shine & Lal ¹³	MCVxMCVxMCH/100	<1530	>1530
Green & King ¹⁴	MCVxMCVxRDW/(Hbx100)	<72	>72
Ricerca ¹⁵	RDW/RBC count	<3.3	>3.3
RDWI ¹⁶	MCVxRDW/RBC	<220	>220

Sensitivity & specificity, and positive & negative predictive values were calculated as follows.²¹

Sensitivity: True positive / (true positive + false negative)
 Specificity: True negative / (true negative + false positive)
 Positive predictive value: True positive / (true positive + false positive)
 Negative predictive value: True negative / (true negative + false negative)
 Youden's index: (sensitivity + specificity) - 100

Table 2: Differential values of discriminatory indices and correctly identified number of patients.

	BTT (n=223)	Non BTT (n=89)	Total correctly diagnosed patients (n=312)	Percentage of correctly identified patients
RDWI				
BTT	207	16	275	88.14
Non BTT	21	68		
Mentzer				
BTT	208	15	271	86.85
Non BTT	26	63		
Green & Kings				
BTT	207	16	262	83.97
Non BTT	34	55		
Srivastava				
BTT	185	38	257	82.37
Non BTT	17	72		
Ricerca				
BTT	178	45	251	80.44
Non BTT	16	73		
England Fraser				
BTT	165	58	238	78.28
Non BTT	16	73		
Shine Lal				
BTT	223	0	226	72.43
Non BTT	86	3		

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

Discriminating index	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Youden's Index
Shine Lal					
BTT	72	100	100	3	72
Non BTT	100	72	3	100	
RDWI					
BTT	91	81	94	76	72
Non BTT	81	91	76	94	
Mentzer					
BTT	89	81	93	71	70
Non BTT	81	89	71	93	
Green & Kings					
BTT	86	78	93	78	64
Non BTT	78	86	78	93	
Ricerca					
BTT	92	62	80	82	50
Non BTT	62	92	82	80	
England Fraser					
BTT	91	56	74	82	47
Non BTT	56	91	82	74	
Srivastava					
BTT	61	66	83	81	27
Non BTT	66	61	81	83	

Serum ferritin estimation was not available so the non BTT group could not be confirmed as iron deficiency anemia.

The following cell counter based formulae were applied in each case.

RESULTS

The differential value for each discrimination index in differentiation between BTT and IDA and the number /percentage of correctly diagnosed/ identified patients calculated are shown in Table 2. This table reveals that percentage of correctly diagnosed patients is highest for RDW index (88.14%) which is closely followed by Mentzer Index (86.85%). The third highest was Green & Kings Index (83.97%). Thus according to percentage correctly diagnosed criteria RDWI and Mentzer are considered as the two best discrimination indices.

The Sensitivity, Specificity, Positive predictive value, Negative predictive value and Youden's Index of each discrimination Index in differentiating between BTT and Non BTT groups are given in Table 3. Analysis of this table indicates that when Youden's Index is enacted as discrimination criteria, once again RDWI is leading. It is followed by Shine and Lal and Mentzer. Combining the above two criteria, it is evident that the two best discrimination are RDWI and Mentzer.

DISCUSSION

Various formulae based on Hb, RBC count, MCV and RDW have been devised in an attempt to separate IDA from BTT as both cause microcytic hypochromic anemia.^{10-16,22} We applied all seven formulas, and found percentage correctly diagnosed is highest for RDW index (88.14%), followed by Mentzer index (86.85%), Green & King (83.97%), Srivastava (82.37%), Ricerca (80.44%),

England & Fraser (78.28%) and Shine & Lal (72.43%) in decreasing order. Similar observations were made by Demir²¹ for the RDWI index (92%), Mentzer Index (76%), Green & King Index (86%), Srivastava (67%), England & Fraser (78%) and Shine & Lal (59%).

In addition to calculate sensitivity and specificity, positive predictive value and negative predictive value for each discrimination index, we also calculated Youdens index in differentiation between BTT and IDA. In the present study Youden's index of RDWI and Shine Lal were the highest followed by Mentzer. Similar Youden Index for RDWI was reported by Demir²¹ and Sirdah.²³ According to Demir²¹ the Youden indices of RBC count and RDWI were the highest 82% and 80% respectively. They concluded that RBC count and RDWI are the most reliable discrimination indices in differentiation between BTT and IDA. While Okan et al²⁴ reported that Shine & Lal and Green & King offered the best discrimination. Similar to our study Okan found that Shine & Lal showed the highest Youden index for BTT and IDA discrimination, but the specificity was not 100%.

Ehsani's study²⁵ of 284 patients showed Mentzer as the best discrimination index. In our study Mentzer's index sensitivity 89% and specificity of 81% were observed. Similar results (sensitivity 90.9% and specificity 80.3%) were found by Ghafouri.²⁶ However none of the indices allowed complete discrimination and similar studies carried out by different authors reported different indices to better distinguish BTT from IDA e.g. Melo²⁷ observed that BTT was better distinguished from IDA by RBC count and England's index. Urrechaga²⁸ and Ntaios²⁹ found that Green and King index ranked the best with a Youden index of 80.9% and 70.86% respectively.

None of the formulas are 100% sensitive and specific, therefore are of importance only for screening in areas of high prevalence of BTT. Successful prevention programs for BTT in Greece and Italy have relied on both screening by RBC indices and Hb A2 concentration.³⁰

Jimenez³¹ assessed the diagnostic value of hematological parameters in differentiating between BTT and IDA and found that when serum iron concentration was included, there was high predictable capacity. The sensitivity and specificity were 86.9% and 80.1% using hematological parameters only, whereas with inclusion of serum iron concentration it rose to 94.2% and 91.6%. Similarly if estimation of ferritin or iron were available our study might have shown a better predictive capacity.

In addition in our population there is a high prevalence of iron, vitamin B12 and folic acid de-

ficiencies along with high incidence of BTT which affects the RBC indices, so we may also have a large number of atypical cases. i.e. patients with both BTT & IDA. To improve the sensitivity and specificity of our results we need to be aware of the possibility of BTT in combination with IDA, in addition to differentiation of the two most common causes of microcytic hypochromic blood picture.

Lack of facility for estimation of serum ferritin was the limitation of our study and IDA could not be confirmed nor was it possible to diagnose cases with both IDA & BTT.

CONCLUSION

Automated cell counters provide a rapid, technically reliable method for screening of BTT cases and cell counter-based formulas particularly RDWI, Mentzer and Shine & Lal have good discriminative function compared with England & Fraser, Green & King and Ricerca. However none of the formulas are 100% sensitive and specific.

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