Original Article

Evaluation of Various Discrimination Indices in Differentiating Iron Deficiency Anemia and Beta Thalassemia Trait: A Practical Low Cost Solution

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ABSTRACT

Background: Mild microcytic hypochromic anemias due to Iron deficiency (IDA) and beta thalassemia trait(β-TT) continue to be a cause of significant burden to the society, particularly in the poorer developing countries. The objective of the present study was to evaluate the validity of 12 different discrimination indices to distinguish β-TT from IDA.

Methods: A total of 225 patients diagnosed with mild microcytic hypochromic anemia on complete blood count and peripheral blood film were included in the study. HB, RBC count, MCV, MCH and RDW obtained from the electronic cell counter were used to calculate 12 discrimination indices by various mathematical formulae. Sensitivity, specificity, positive predictive value(PPV), negative predictive value(NPV) and Youden's index(YI) were calculated.

Result: The Shine & Lal and Ricerca et al indices exhibited the highest sensitivity of 100% each while the England & Fraser index demonstrated the lowest sensitivity of 61.1%. The England & Fraser and Mentzer indices demonstrated the highest specificities of 90.2% and 86.9% respectively. The highest and the lowest PPV were found for Mentzer index (77.3%) and MCHD (32.71%) respectively. The Ricerca et al. and Shine & Lal indices demonstrated the highest NPV of 100% each and the lowest NPV was exhibited by MCHD (69.8%). The highest and the lowest values for Youden's index were shown by Mentzer index (81.3%) and MCHD (2.36%) respectively.

Conclusion: Though HBA2 estimation is the gold standard for diagnosing β-TT, in developing countries, Mentzer index followed by Ehsani et al. index can be used to screen mild microcytic hypochromic anemia cases to eliminate as many false positive cases as possible to reduce the financial cost.

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Introduction

Iron deficiency anemia (IDA) and beta thalassemia trait (β-TT) are the two most frequent disorders presenting clinically with mild microcytic hypochromic anemia.[1] Lack of sufficient dietary iron resulting in IDA is the most common hematological disorder. It has been estimated that 30% of the world population suffers from IDA with majority of the affected people living in developing countries. In beta thalassemia trait there is impaired globin chain synthesis resulting in decreased hemoglobin leading to microcytic hypochromic anemia. 1.5% of world population carries genes for beta thalassemia.[2]

Thalassemia traditionally has a high prevalence in some parts of the world (Mediterranean regions, up to 8%; countries of the Middle East, up to 10%;India, 3%-15%; Southeast Asia, up to 9%), where it represents a major public health problem. Non endemic countries such as in Northern Europe and North America are also involved in thalassemia related problems as a result of demographic changes caused by migration and intermarriages of different ethnic populations.[3,4] Nowadays population migration has spread thalassemia genes over nearly the entire globe.[5] In India, certain communities such as Sindhis, Kutchis, Lohanas, Bhanusalis, Punjabis, Mahars, Agris, Gauds, Saraswats, Gowdas etc. have a higher frequency.[6]

It is important to differentiate between thalassemic and nonthalassemic microcytosis as both conditions share many characteristics and have important clinical implications.[7] Thus a correct diagnosis in patients with microcytic anemia can provide an indication for supplementing iron to IDA patients, for avoiding unnecessary iron therapy in thalassemia carriers and of course also for preventing severe and lethal forms of thalassemia syndromes in the framework of premarital counseling in high-prevalence areas.[8]

A definitive differential diagnosis between β-TT and IDA is based on the result of HBA2 electrophoresis, serum iron levels, and ferritin calculations.[9] However, these investigations are money and time consuming and moreover areas where thalassemia is endemic often have low health care resources and these assays may not be generally available.[8]

Thus, various discrimination indices have been proposed to distinguish between β-TT and IDA. These indices are derived from several simple red blood cell (RBC) indices, like RBC count, mean cell volume (MCV), mean corpuscular hemoglobin (MCH), RBC distribution width (RDW) and hemoglobin (HB) as these are provided by electronic cell counters.[10] The purpose of using indices to discriminate anemia is to detect subjects who have a high probability of requiring appropriate follow-up and to reduce unnecessary investigative costs. An ideal discrimination index has high sensitivity and specificity; that is, it can detect the maximum number of patients with β-TT (high sensitivity) while eliminating patients with IDA (high specificity).

Not much work is available from Northern India where the incidence of β-TT is high (3-15%). [3,4] The present study was undertaken, to evaluate the ability of 12 different discrimination indices to distinguish β-TT from IDA by calculating their sensitivity, specificity and Youden's index values in a population group of North India and to compare its findings with studies done in other parts of the world.

Materials and Methods

The present study was conducted in the department of Hematology in a tertiary health care center in North Indian state of Punjab. A total of 225 patients (167 children and 58 adults) attending the outpatient departments of various specialties and diagnosed with mild microcytic hypochromic anemia on complete blood count and peripheral blood film were included in the study. Family members (Parents and siblings) of known cases of beta thalassemia major coming for blood transfusion of the affected child were also included in the study group. They were selected because parents of a beta thalassemia major patient carry beta thalassemia trait gene (100%) and the chances of carrying this gene in the siblings is also 50%.

Venous blood was drawn with the usual precautions after the patient had been lying quietly for at least 20 minutes. The hematological analyses were carried out with a Sysmex XS-800i particle counter. HB concentration, RBC count, MCV, MCH and RDW were recorded. High performance liquid chromatography technique was used for determination of HbA2 [11] Serum iron (SI), serum iron binding capacity (SIBC), transferrin saturation (TS), serum ferritin, and HbA2 values were determined in all. The mean values and standard deviation of various hematological and biochemical parameters are shown (Table 1).

Patients with hemoglobin concentration between 8.6-11.5g% and MCV less than 80fl were included in the study. Those having HB less than 8.6g% were excluded. The cut off value for HBA2 was kept at 3.5%. Patients with HBA2 levels more than 3.5% were labelled as β-TT group and those with value less than 3.5% were labelled as IDA group.

Values of HB, RBCcount, MCV, MCH and RDW obtained from the electronic cell counter were used to calculate 12 discrimination indices by various mathematical formulae

as given in (Table2). Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and Youden's index (YI) were calculated for all the 12 discriminants as follows:

Sensitivity = True Positive / True Positive+False Negative × 100.

Specificity=True Negative/TrueNegative+FalsePositive × 100.

PPV = Truepositive / True Positive+FalsePositive × 100.

NPV = True Negative/ True Negative+ False Negative × 100.

Youden's index = (sensitivity+specificity) – 100.

P values less than 0.05 were considered significant.

The differential values for each discrimination index were applied as defined in the original published reports. [12-20] The number and proportions of correctly identified patients (True positive) were calculated (Table 3). Sensitivity, specificity, PPV, NPV and Youden's index for each discrimination index were calculated (Table 4).

Result

In the present study, the maximum number of patients belonged to the 5-15 years age group (134, 59.5%) with range being 1.5- 39 years. Majority of the patients in the present study had no obvious complaints related to anemia (57%) but weakness (38%) followed by fatigue (5%) were the next common complaints received. On the basis of HBA2 levels, 72 patients having HBA2 more than 3.5% were grouped into β-TT group. Rest 153 patients having HBA2 less than 3.5% were included in the IDA group. The mean values for HB in β-TT group were 10.7± 0.8 and those in the IDA group were 9.9± 1.43 (p>0.05; not significant).

In β-TT group, the mean MCV and MCH were 63.6±5.49 and 18.4±2.15 respectively which were lower than those found in the IDA group (69.4±5.16 and 20.67±1.34 respectively). (p<0.05; significant). The mean RBC count in β-TT group was definitely higher 5.79±0.76 as compared to the IDA group 4.77±0.53. (p<0.05; significant). The mean RDW values were increased in both the groups, but the IDA group showed the values to be higher (17.2±2.96) as compared to the β-TT group (15.9±1.62). (p>0.05; not significant)

The mean values for serum iron, transferrin saturation and serum ferritin were much lower in IDA group as compared to those in the β-TT group but TIBC showed an inverse trend. [Table 1]. As is evident from the results shown in Table 4; none of the indices showed 100% efficiency in recognizing beta thalassemia trait. The Shine and Lal and Ricerca et al indices exhibited the highest sensitivity of 100% each but failed miserably when it came to specificity which was very low to the tune of 6.25% and 16.9% respectively.

The England and Fraser index demonstrated the lowest sensitivity of 61.1% which means it missed about 39% of β-TT cases. The England and Fraser and Mentzer indices demonstrated the highest specificities of 90.2% and 86.9% respectively.

The highest and the lowest PPV were found for Mentzer index (77.3%) and MCHD (32.71%) respectively. The Ricerca et al. and Shine and Lal indices demonstrated the highest NPV of 100% each and the lowest NPV was exhibited by MCHD (69.8%). The highest and the lowest values for Youden's index were shown by Mentzer index (81.3%) and MCHD (2.36%) respectively. None of the indices was completely sensitive and specific in differentiating between BTT and IDA.

Table I: Showing the Mean Values and Standard Deviation of Various Hematological and Biochemical Parameters.

S. No.	Parameter	β-TT		IDA	
		Range	Mean± SD	Range	Mean± SD
1.	Hb	9-11.5	10.7±0.8	8.6-11.3	9.9±1.43
2.	RBC	4.6-6.4	5.79±0.76	3.7-5.3	4.77±0.53
3.	MCV	56.4-71.1	63.6±5.49	54.2-78.4	69.4±5.16
4.	MCH	16.1-22.4	18.4±2.15	14.2-26.7	20.67±1.34
5.	RDW	14-22.9	15.9±1.62	12.7-27.1	17.2±2.96
6.	SI	26.1-178	78.2±18.3	5.3-27.4	21.1±9.4
7.	SIBC	261-398	312±26.2	279-473	386±38.1
8.	TS	7.1-62	23.7±7.1	1.1-7.9	6.4±2.1
9.	S Ferritin	14.7-87	36.1±18.4	2.3-12.8	6.92±2.9

Table 2: Showing Different RBC Indices and Mathematical Formulae Used to Differentiate Between B-Tt and Ida.

Hematological index	Year	Formula
Mentzer index	1973	MCV/RBC
England and Fraser Index	1973	MCV − (5 × Hb) − RBC − 3.4
Srivastava Index	1973	MCH/RBC
Shine and Lal Index	1977	MCV × MCV × MCH/100
RDW Index	1987	MCV × RDW/RBC
Ricerca et al Index	1987	RDW/RBC
Green and King Index	1989	MCV × MCV × RDW/Hb × 100
MDHL Index (Mean Density of Hb/liter of Blood)	1999	(MCH/MCV) × RBC
MCHD Index (Mean Cell Hb Density)	1999	MCH/MCV
Sirdah Index	2007	MCV − (5 × Hb) − RBC − 3.4
Ehsani et al Index	2009	MCV − (10 × RBC)

Table 3: Showing Number of Correctly Identified Patients By Each Discrimination Index.

S.No.	INDICES	β-TT	IDA	TOTAL No. of Correctly Diagnosed Cases	PERCENTAGE
1.	Mentzer β-TT<13 IDA>13	68 04	20 133	68+133=201	89.3
2.	RBC β-TT>5 IDA<5	67 05	55 98	67+98=165	73.3
3.	England & Frazer β-TT<0 IDA>0	44 28	15 138	44+138=182	80.9
4.	Srivastva β-TT<3.8 IDA>3.8	63 09	55 38	63+38=101	44.9
5.	Shine & Lal β-TT<1530 IDA>1530	72 00	144 09	72+9=81	36.0
6.	RDWI β-TT<220 IDA>220	58 14	24 119	58+119=177	78.6
7.	Ricerca et al β-TT<4.4 IDA>4.4	72 00	127 26	72+26=98	43.6
8.	Green & King β-TT<65 IDA>65	57 15	46 107	57+107=164	72.9
9.	MDHI β-TT>1.63 IDA<1.63	54 18	70 83	54+83=137	60.9
10.	MCHD β-TT>0.3045 IDA<0.3045	53 19	109 44	53+44=97	43.1

S.No.	INDICES	β-TT	IDA	TOTAL No. of Correctly Diagnosed Cases	PERCENTAGE
11.	Sirdah β-TT<27 IDA>27	54 18	25 128	54+128=182	80.9
12.	Ehsani et al β-TT<15 IDA>15	67 05	26 127	67+127=194	86.2

Table 4: Showing Sensitivity, Specificity, Positive Predictive Value (Ppv), Negative Predictive Value (Npv), and Youden's Index for all Twelve Discrimination Indices Between B-Tt and Ida.

S.No.	INDICES	SENSTIVITY	SPECIFICITY	PPV	NPV	Youden's Index
1.	Mentzer β-TT IDA	 94.4% 86.9%	 86.9% 94.4%	 73.3% 97.1%	 97.1% 77.3%	81.3%
2.	RBC β-TT IDA	 93.05% 64.05%	 64.05% 93.05%	 54.9% 95.1%	 95.1% 54.9%	57.1%
3.	England & Frazer β-TT IDA	 61.1% 90.0%	 90.2% 61.1%	 76.6% 83.1%	 83.1% 76.6%	51.3%
4.	Srivastva β-TT IDA	 87.5% 64.05%	 64.05% 87.5%	 53.4% 90.7%	 90.7% 53.4%	51.55%
5.	Shine & Lal β-TT IDA	 100% 6.25%	 6.25% 100%	 33.3% 100%	 100% 33.3%	6.25%
6.	RDWI β-TT IDA	 80.5% 77.7%	 77.7% 80.5%	 70.7% 89.47%	 89.47% 70.7%	58.2%
7.	Ricerca et al β-TT IDA	 100% 16.9%	 16.9% 100%	 39.2% 100%	 100% 39.2%	16.9%
8.	Green & King β-TT IDA	 79.2% 69.9%	 69.9% 79.2%	 55.3% 87.7%	 87.7% 55.3%	49.1%
9.	MDHI β-TT IDA	 75.0% 54.24%	 54.24% 75.0%	 43.54% 82.17%	 82.17% 43.54%	29.4%
10.	MCHD β-TT IDA	 73.61% 28.75%	 28.75% 73.61%	 32.71% 69.84%	 69.84% 32.71%	2.36%
11.	Sirdah β-TT IDA	 75.0% 85.65%	 83.65% 75.0%	 68.4% 87.6%	 87.6% 68.4%	58.65%
12.	Ehsani et al β-TT IDA	 93.05% 83.0%	 83.0% 93.05%	 72.04% 96.2%	 96.2% 72.04%	76.05%

Discussion

Correct identification of β-TT is especially important, as the management of a patient with beta thalassemia major is not only expensive (strains on the limited resources of developing countries),[21] but also causes extreme misery to the patient and the family due to compromised quality of life. Also as the red cell morphology in β-TT is microcytic hypochromic; these patients are often misdiagnosed as those suffering from IDA and given unnecessary iron medication. In this setting a reliable discrimination index becomes a need of the hour.

The best index for beta thalassemia trait should have a very high sensitivity as well as a reasonably high specificity so that the number of false positive cases can be reduced to minimum.

Since 1973, several indices have been introduced in an attempt to distinguish these two conditions in a cheaper and easier way. According to the original published papers by the authors of these indices, their sensitivity in the detection of β-TT from IDA is approximately 100%.[12,13,17,22-26] However later studies failed to confirm these results and estimated these indices' sensitivity to be between 61-91%.[27-31] These indices incorporate MCV, MCH, RBC Count, RDW and HB in various combination. Table I shows that there were significant differences between the haematological and biochemical parameters of β-TT and IDA but these did not reflect in the indices' reliability in differential diagnosis of β-TT and IDA.

RBC count has been considered as a valuable index.[32] Majority of the β-TT patients in the present study had a high RBC count of more than 5 million. However a significant number of IDA patients (55/153) also had an RBC count of >5 million. So though the sensitivity of RBC count for beta thalassemia trait was 93.05%, it showed a specificity and Youden's index of only 65.05% and 57.1% respectively. So according to our results, RBC count alone cannot be taken as a reliable index to distinguish between of beta thalassemia trait and IDA.

RDW, a measure of the degree of variation in red cell size, has been reported to be a good discrimination index to differentiate between β-TT and IDA[33-36]. But in our study the values were elevated in both the groups, though they were slightly higher in the IDA group. In the present study, RDW index showed the sensitivity of 80.5%, specificity of 77.7 % and youden's index of 58.2%. Our results concur well with results of[37-39] who found that RDW alone is reasonable but not sufficiently specific or sensitive enough to differentiate between β-TT and IDA.

In the present study, the Mentzer index showed a reasonably high sensitivity, specificity and good Youden's index of 94.4%, 86.9% and 81.3% respectively. This was followed by Ehsani et al index which showed sensitivity, specificity and Youden's index of 93.05%, 83.0% and 76.05% respectively. Ehsani et al. in 2009 showed that the best discrimination index according to Youden's criteria was the Mentzer index (90.1%), followed by the Ehsani et al.index(85.5%). In their study, the Mentzer and Ehsani et al. indices were able to correctly diagnose 94.7% and 92.9% of cases, respectively.[20]

Rahim and Keikhaei in 2009 examined the diagnostic accuracy of 10 indices in 153 patients with β-TT and 170 patients with IDA. They found that the Mentzer index had 85% sensitivity, 93% specificity, and 79% Youden's index.[40] Similar results (Mentzer index: sensitivity, 90.9%;specificity,80.3%)were found by Ghafouri et al**.** [41]

In 2007, AlFadhli et al.[42] in their research work applied and compared nine well-documented discriminant functions in 153 confirmed cases of microcytic anemia and measured validity using Youden's index. They showed that the England & Frazer index had the highest Youden's index value (98.2%) in correctly differentiating between IDA and α - and ß-thalassemia minor, while the Shine & Lal index was found ineffective in differentiating between microcytic anemias in their study. Ferrara et al. in 2010 demonstrated that the England and Fraser index had the highest specificity of 99.1 % and a Youden's index of 64.2%.[43]

In the present study, the England and Frazer index had the highest specificity of 90.2 %, but the Youden's index was 51.3%. Shine and Lal index showed a Youden's index of only 6.25% and proved to be ineffective in differentiating between β-TT and IDA. In the present study, the Sirdah index demonstrated a sensitivity, specificity and youden's index of 75.0%, 83.6% and 58.6% respectively. The findings are comparable with the results obtained by Vehapoglu et al 2014[44] who found these to be 85.7%, 79.4% and 65.0% respectively. In the present study, the Srivastva index showed a sensitivity, specificity and Youden's index of 87.5%, 64.05% and 51.5% respectively. Alfadhli et al compared nine discriminant functions in patients with microcytic anemia and found a Youden's index of 54.9% for Srivastva index.[42]

In 2007, Ntaios et al. reported that the Green and King index was the most reliable index, as it had the highest sensitivity (75.06%), efficiency (80.12%), and Youden's index (70.86%) for detecting β-TT.[10] A similar result for the Green and King index (Youden's index, 80.9%) was found by Urrechaga et al.[45] However in the present study, though the sensitivity was 79.2%; it showed a low specificity and Youden's index of 69.9% and 49.1% respectively. The

Ricerca index also exhibited a sensitivity of 100% but the specificity was very low (16.9%) and a Youden's index of 16.9% ruling it out as a reliable indicator. Similar results were obtained by Vehapoglu et al 2014[44] who found a youden's index of 14.7%.

In the present study, the MDHL index showed a sensitivity of 75.0% and a youden's index of 29.4%. The MCHD index exhibited a very low Youden's index of 2.36% only. These findings are in coherence with the works done by Vehapoglu et al 2014.[44] So in the present study, according to the Youden's index which is a measure of high performance of the discrimination index, following rankings of various discrimination indices were obtained. Mentzer> Ehsani> Sirdah> RDWI> RBC> Srivastva> England & Fraser> Green & King> MDHL> Ricerca> Shine & Lal> MCHD.

However one important point needs a special mention in β-TT. Literature documents more than 200 mutations noted in β-TT so far, and also according to the extent of the reduction of beta chain output, these mutations have been divided in to severe, mild and silent.[46] It is these silent mutations (measurable only by special genetic studies) which are characterized by near normal hematological indices and borderline HBA2 levels leading to mislabeling of these individuals as phylogenetically normal which may result in genotypic abnormalities in the next generation leading to much avoidable morbidities. But these high end and expensive genetic studies are not widely available in resource challenged countries where applications of these hematological indices are practically implementable.

Conclusion

From the above data, it is clear that Mentzer index followed by Ehsani et al. index are highly sensitive and reasonably specific in differentiating β-TT from IDA and none of the indices is 100% sensitive and specific. Though HBA2 estimation continues to be the gold standard for diagnosing a case of β-TT, a practical approach in developing countries with limited health care resources would be to screen mild microcytic hypochromic cases by these 2 indices and try to eliminate as many false positive cases as possible to reduce the financial cost.

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Competing Interests

None Declared

Reference

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