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EVALUATION OF DIAGNOSTIC ACCURACY OF HEMATOLOGICAL INDICES IN THE DIFFERENTIATION OF BETA THALASSEMIA TRAIT FROM IRON DEFICIENCY ANEMIA

Pathology

Dr. Kavita Choudhary* *Corresponding Author
Dr. M. L Gupta
Dr. Monika Hemin Shroff
Dr. Abhishek Rajawat

ABSTRACT

Background: 2 most frequent type of microcytic hypochromic anemias are Iron deficiency anemia (IDA) and beta thalassemia trait (β-TT) continue to be a cause of significant burden to the society, particularly in developing countries like INDIA, where there is limitation of resources. The objective of the present study was to evaluate the validity of different discrimination indices to differentiate β-TT from IDA.
Methods: All diagnosed patients of microcytic hypochromic anemia admitted in Geetanjali Medical College and Hospital, Udaipur (Raj) between Jan 2018 to June 2020, were included in this study. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and Youden's index (YI) were calculated.
Result: According to our study following rankings of various discrimination indices were obtained for BTT patients, RDW Index > Mentzer's Index = Ehsani Index > Sirdah Index > MDHL Index > MCHD Index.
Conclusion: According to our results and observations, the percentage of correctly diagnosed patients are the highest with RDW Index and Mentzer's Index. It should be kept in mind that several studies have shown that none of the red cell indices carry 100% sensitivity and specificity in diagnosing BTT.

KEYWORDS

Red blood cell indices, microcytic hypochromic anemia, iron deficiency anemia, B-thalassemia trait.

INTRODUCTION

Anemia is defined as reduction of the total circulating red cell mass below normal limits for same age group and sex. 30% of world's population might be affected by anemia as estimated by world health organizations. On the basis of RBC morphology, anemia is further divided in 3 types: normocytic normochromic, microcytic hypochromic and macrocytic normochromic.[1]

Iron deficiency anaemia (IDA) and Beta Thalassaemia Trait (BTT) are two different types of anaemias, where both show microcytic morphology of RBC.[2]

Microcytic Hypochromic anemia is characterized by low MCV (Mean Corpuscular Volume) and low MCH (Mean corpuscular hemoglobin).[3] Iron deficiency anemia is characterized by microcytic hypochromic red cells with low serum iron and low serum ferritin. Morphologic changes of red cells appear as the iron stores get depleted and iron is not available in adequate amounts for hemosynthesis.[2,4]

Beta thalassemia trait is a type of hemolytic anemia which is also microcytic hypochromic. It is heterozygous genetic disease in which there is defect in synthesis of Beta globin chains.

Basic criteria of difference between BTT and IDA, as given by the earlier scientists, was that in BTT, MCV is never less than 70fl, RBC count is normal and Hb% is around 10 gm% whereas in IDA, MCV is much lower, RBC count is low and Hb% is also low. Later on, with the introduction of cell counters, RDW was also incorporated.[5]

BTT is a haemoglobinopathy, a congenital disease whereas IDA is an acquired disease, which is mainly due to dietary deficiency of iron. BTT can't be prevented or cured but iron deficiency anaemia can be prevented and cured by supplementing iron. ß-thalassaemia trait presents with microcytic hypochromic blood picture similar to the picture of iron deficiency anemia, but both the disease have different diagnostic, prognostic and treatment protocol.

Confirmatory test for BTT is HbA2 level more than 3.5% and for IDA is low iron, high TIBC and low iron saturation. So the differentiation between these conditions becomes mandatory which usually relies on the measurement of serum ferritin, serum iron, and total iron binding capacity and HbA2 levels.[5]

These diagnostic methods are costly and hence constitute a significant burden on public health economy particularly in developing countries like India. This made the way for developing a simple and economic screening test for the detection of carriers of ß-thalassemia. So, in order to differentiate IDA from BTT, different Red Cell indices are taken into account.[6]

6 Different types of discrimination indices have been used in our study like:
Mentzer index is ratio of MCV/TRBC count. If the quotient is less than 13 means more red blood cells are being produced by marrow to compensate anemia. This finding is most likely seen in B-thalassemia trait. If the quotient is greater than 13, means less numbers of red cells are being produced by marrow. This is most likely seen in iron deficiency anemia.[7]

RDWI is calculated as MCV×RDW/TRBC. If the index is <220, the patient is tabulated as βTT patient, while values >220 classified as IDA.[7]

Sirdah index is calculated as MCV − TRBC − (3 × Hb). If the index is <27, the patient is tabulated as βTT patient, while values >27 classified as IDA.[7]

Ehsani index is calculated as MCV − (10 × TRBC). If the index is <15, the patient is tabulated as βTT patient, while values >15 classified as IDA.[7]

MDHL index is calculated as (MCH/MCV) × TRBC. If the index is >1.63, the patient is tabulated as βTT patient, while values <1.63 classified as IDA.[7]

MCHD index is calculated as MCH/MCV. If the index is >0.3045, the patient is tabulated as βTT patient, while values <0.3045 classified as IDA.[7]

MATERIAL AND METHODS:

It was observational study, conducted in the Department of Pathology, GMCH, Udaipur. From January 2018 to June 2020, a total of 83 patients were included in this study. Purposive consecutive sampling technique was used. All diagnosed patients of microcytic hypochromic B-thalassemia trait and iron deficiency anemia were included in this study.

Exclusion Criteria:
Cases having other types of anemia, patients on blood transfusion, patients whose Hb-electrophoresis is not done.

Whole blood samples of patients were used. The samples were collected in 3 different vials, 2 containing EDTA and 1 vial without anticoagulant, and were analyzed within two hours of collection, using automated hematology analyzers (ABX PENTRA XL 80), biochemical analyzer (COBAS C311, COBAS E411) and automated electrophoresis apparatus(BIO RAD D10). Each sample was sent for CBC, iron profile and Hb-electrophoresis.

Following formulas used in our study:
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.

Statistical Analysis:
The data was entered in MS Excel software version 17 and analyzed using SP5S, IBM Comp. version 21. The descriptive data was expressed in proportions, mean and frequency tables. The categorical data were analyzed using Chi-square test. The quantitative data was analyzed using independent Student's T-test. P value less than 0.05 was considered statistically significant. The differential values for each index was applied as defined in original published reports.

RESULTS:
There is more number of males (54.22%) than females (45.78%) in our study. Majority of the patients are in the younger age group of <30 years (73.48%). 48 (57.83%) of patients were of IDA and 35 (42.17%) patients of BTT. It suggests IDA is more prevalent than BTT in society. On comparing the mean and SD of different haematological parameters of IDA & BTT (**TABLE 1**). Mean RBC is significantly more in BTT (5.73 ± 0.61) as compared to IDA (3.87 ± 0.95). Mean hemoglobin (10.13 gram/dl) is also more in BTT cases as compared to IDA (7.04 gram/dl). Similarly, RDW (16.43%) is lower when compared to IDA. Statistically MCV and MCH do not show much difference; therefore it becomes very difficult to differentiate BTT and IDA on the basis of MCV and MCH. Since TRBC and HCT are significantly different in IDA and BTT, most of the calculated indices use these values. The mean and SD of different biochemical parameters of IDA & BTT are significantly different. Biochemical markers like S. iron and S. ferritin are significantly low in IDA group as compared to BTT, whereas TIBC is significantly high in IDA group as compared to BTT group ($p<0.001$).

Table 1: Showing The Mean Values And Standard Deviation Of Various Hematological And Biochemical Parameters.

S. No.	Parameter	IDA		BTT	
		Mean	± SD	Mean	± SD
1.	Hb	7.04	2.37	10.13	1.91
2.	RBC	3.87	0.95	5.73	0.61
3.	MCV	61.58	5.78	57.83	7.45
4.	MCH	17.79	2.91	17.64	2.66
5.	RDW	20.35	3.45	16.43	2.50
6.	SI	20.65	6.22	98.06	23.39
7.	TIBC	495.46	62.69	304.95	46.54
8.	S Ferritin	8.22	3.42	70.06	26.26

Mentzer's index and Ehsani index are showing highest accuracy (100%) for BTT while MDHL index (100%) and Sirdah index are better for IDA (**TABLE 2**). 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.

Table 2: Efficiency Of Haematological Indices In Diagnosis Of IDA And BTT

HAEMATOLOGICAL INDICES	IDA (N=48)		BTT (N=35)	
	IDENTIFIED CORRECTLY	PERCENTAGE	IDENTIFIED CORRECTLY	PERCENTAGE
Mentzer's Index	42	87.5%	35	100%
RDW Index	46	95.83%	33	94.28%
Sirdah Index	47	97.91%	25	71.42%
Ehsani Index	42	87.5%	35	100%
MDHL Index	48	100%	23	65.71%
MCHD Index	32	66.66%	20	57.14%

Youden's index is maximum (90%) with RDW index followed by Mentzer's index and Ehsani index (88%), Sirdah index (69%), MDHL index (66%) and MCHD index (24%) in different Haematological Indices in IDA and BTT patients**.** While Sensitivity, Specificity, PPV, NPV were different for different indices (**TABLE 3).**

Table 3: Showing Sensitivity, Specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV), And Youden's Index For IDA And BTT.

S.No.	INDICES	SENSTIVITY	SPECIFICITY	PPV	NPV	Youden's Index
1.	Mentzer index					
	β-TT	100%	88%	85%	100%	88%
	IDA	88%	100%	100%	85%	
2.	RDW index					
	β-TT	94%	96%	94%	96%	90%
	IDA	96%	94%	96%	94%	
3.	SIRDAH index					
	β-TT	71%	98%	96%	82%	69%
	IDA	98%	71%	82%	96%	
4.	EHSANI index					
	β-TT	100%	88%	85%	100%	88%
	IDA	88%	100%	100%	85%	
5.	MDHL index					
	β-TT	66%	100%	100%	80%	66%
	IDA	100%	66%	80%	100%	
6.	MCHD index					
	β-TT	57%	67%	56%	68%	24%
	IDA	67%	57%	68%	56%	

DISCUSSION:
Both BTT and IDA have an entirely different cause, prognosis, and treatment. Hence, distinguishing them has important clinical implications. However, no single marker or any combination of tests has been found to be optimal for this discrimination. Upto now, many investigators have used different mathematical indices to distinguish BTT from IDA using only a complete blood count. This process helps to select appropriate individuals for a more detailed examination; however, no study has found 100% specificity or sensitivity for any of these RBC indices.

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 BTT from IDA is approximately 100%. However later studies failed to confirm these results and estimated these indices' sensitivity to be between 61-91%. These indices incorporate MCV, MCH, RBC Count, RDW and HB in various combinations.

In our study according to Mentzer's Index, Sensitivity is 100%, Specificity 88%, PPV 85%, NPV 100% and Youden's Index is 88% in BTT patients. According to RDW Index, Sensitivity is 94%, Specificity 96%, PPV 94%, NPV 96% and Youden's Index was 90% in BTT patients. According to Sirdah Index, Sensitivity is 71%, Specificity 98%, PPV 96%, NPV 82% and Youden's Index was 69% in BTT patients. According to Ehsani Index, Sensitivity is 100%, Specificity 88%, PPV 85%, NPV 100% and Youden's Index is 88% in BTT patients. According to MDHL Index, Sensitivity is 66%, Specificity 100%, PPV 100%, NPV 80% and Youden's Index is 66% in BTT patients. According to MCHD Index Sensitivity is 57%, Specificity 67%, PPV 56%, NPV 68% and Youden's Index is 24% in BTT patients.

Piplani et al[8] (2016) in their study: A total of 225 patients included and 12 discrimination indices were calculated. 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.

Arora S et al[9] (2017) evaluated the reliability of three indices. The Mentzer index was the most reliable index, as it had the highest sensitivity (97.62%), specificity (66.67%), and Youden's index (64%) for detecting Iron deficiency anaemia; the Matos and Carvalho Index showed higher sensitivity (98.81%) but a much lower specificity (33.3%) and Youden's index (32%) while the red cell distribution width index showed the sensitivity and specificity of 92.86% and 66.67%, respectively with Youden's index of 59%.They concluded that the Mentzer index provided the highest reliabilities for differentiating IDA from β-TT.

Bhushan R et al[10] (2018) concluded that sensitivity of Shine and Lal index was highest (98.4%) followed by Kerman 1 index (66.7%). Mentzer index, England and Fraser index, RDW index and Ehsani index each, showed high specificity (99.66%). Kerman 1 index could point towards the correct diagnosis in 97.5% of the patients. Youden index was highest for Kerman 1 index (65.4) followed by RDW index (63.1). None of the index was 100% sensitive or specific.

Valiya K R et al[11] (2019) found, Youden's Index showing validity to differentiate between β-TT and IDA in decreasing order was found as: Ricerca Index (74.57) > RDWI Index (72.73) >Mentzer Index (61.42) > Ehsani Index (56.42) > Green and King Index (54.78) >Sirdah Index (51.63) > Matos and Carvalho Index (51.57) > England and Fraser Index (45.26) > Srivastava Index (42) > MCHD Index (38.89) > Kerman 1 Index(36.84) > MDHL Index (20) > Shine andLal Index (3.84).They concluded that the percentage of correctly diagnosed patients was highest (87.69%) with Ricerca Index closely followed by RDWI Index (87.18 %). The third highest one was Mentzer Index (81.54%).

It can be emphasize that with the easier availability of basic automation in haematology, red cell indices have become fairly sensitive, specific, reproducible and precise and can be reliably used in the peripheral health centers, where expensive investigations like electrophoresis and HPLC are not available. Moreover, it can also minimize the expenditure during mass screening of BTT.

So in our study, according to Youden's index which is a measure of high performance of the discrimination index, following rankings of various discrimination indices were obtained for BTT patients. RDW index > Mentzer index = Ehsani index > Sirdah index > MDHL index >MCHD index.

CONCLUSION:

Youden's index which is a measure of performance of the discrimination index, we calculate the following rankings of various discrimination indices for BTT patients. RDW Index > Mentzer's Index = Ehsani Index > Sirdah Index > MDHL Index > MCHD Index.

According to our results and observations, the percentage of correctly diagnosed patients are the highest with RDW Index and Mentzer's Index.

It should be kept in mind that several studies have shown that none of the red cell indices carry 100% sensitivity and Specificity in diagnosing BTT.

HPLC or chromatography is not available at the peripheral health care system in India. Therefore adequate utilization of combination of these indices can facilitate identification of the majority of BTT cases at no additional cost.

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CONFLICT OF INTEREST:

None declared.

Ethical Approval:

The study was approved by the institutional ethics committee.

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