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ORIGINAL ARTICLE

Role of Hematological Indices as a Screening Tool of Beta Thalassemia Trait in Eastern Uttar Pradesh: An Institutional Study

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Abstract A large majority of microcytic hypochromic anemia have defects in cellular hemoglobin synthesis due to either iron deficiency or thalassemia trait; both differing in management and prognosis. HPLC and serum iron profile as confirmatory tests are unavailable at health care centers. Blanket therapy of iron supplements is therefore given in all such cases which may cause iron overload in thalassemia cases. Easy to use and cost effective screening methods are desirable. The present study was undertaken to evaluate the diagnostic accuracy of twelve indices to effectively screen cases of thalassemia trait and differentiate them from iron deficiency anemia. Routine samples from the hematology lab with Hb < 13 gm/dl, MCV $<$ 80 fl and MCH < 27 pg were screened. Taking HPLC and serum ferritin as gold standard, out of total 1353 cases, 98 cases of thalassemia trait (HbA2 > 3.5 on HPLC) and 1102 cases of iron deficiency anemia (serum ferritin < 12 g/ml) were evaluated using discrimination indices. Diagnostic accuracy for each index was calculated. While few indices showed a sensitivity of 100%, their specificity was low which meant more number of false positive cases. Based on Youden's Index, which measures the diagnostic tests ability to balance sensitivity and specificity, the best three indices in the decreasing order of their efficacy in our study were Ricerca Index (RI), Green and King Index (GKI) and Mentzer Index (MI). MI is considered a reliable index by many clinicians since a long time, however RI and GKI were found to have a better diagnostic accuracy based on our study.

Keywords Anemia · Thalassemia · HPLC · Mentzer

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Introduction

Anemia is a widespread cause of morbidity globally. In a developing country, like India, Iron deficiency anemia (IDA) is a common health problem affecting both rural and the urban populations. Various health programs have been implemented for the prevention of IDA focusing on routine iron supplementation.

Hemoglobinopathies especially Thalassemia constitutes a major cause of hematological morbidity, especially in childhood. The worldwide prevalence of Hemoglobinopathies carrier is estimated to be approximately 7% (ranging from 1 to 20%). The frequency of beta thalassemia in India have been reported from < 1 to 17%, with an average of 3.3% [1] in the general population wherein Beta thalassemia trait (BTT) is the most prevalent condition. The prevalence of BTT in different regions of

India show wide variations: 6.5% in Punjab, 8.4% in Tamilnadu, 4.3% in south India, and 3.5% in Bengal [2].

BTT and IDA despite having a similar morphology differ completely in terms of management and prognosis. IDA requires iron supplements for a prolonged period to replenish the stores while the same may cause iron overload and related complications in thalassemia. A definite diagnosis is made based on High performance liquid chromatography (HPLC) and serum iron profile which may not be affordable by the majority of the population. A blanket therapy of iron supplements is therefore given in all cases causing deleterious effects in thalassemia cases. To avoid expensive and time-consuming procedures for discrimination between the two, studies have evaluated RBC parameters like MCV, MCH, RDW, or formulas derived from these.

Screening of thalassemia carrier cases helps to prevent the birth of a child with Thalassemia major. As these diseases require long-term care, prevention of the homozygous state constitutes major weaponry in the management. It further helps to segregate cases where a complete clinical workup is mandatory for diagnosis and treatment. With adequate screening, genetic counseling of couple can be done when the family is at risk of birth of a child with severe disease.

Materials and Methods

A cross sectional study was conducted in the department of Pathology from October 2017 to September 2019. A total of 1353 samples of patients > 18 years of age with a microcytic hypochromic anemia were analyzed. Routine blood samples with Hb < 13 gm/dl in males and < 12.5 gm/dl in females, MCV < 80 fl and MCH < 27 pg were evaluated. Patients with a clinical suspicion or a family history of hemoglobinopathy were also included while those with history of blood transfusion within 30 days and patients on hematinics were excluded. HbA2 > 3.5% using HPLC and Serum Ferritin of < 12 gm/dl were considered gold standard for BTT and IDA respectively.

Complete Hemogram was done on semi Automated Three part Hematology Analyser (Medonic M 20 series) which was calibrated with commercially available controls. The automated counter functions on the basis of optical scatter. The parameters recorded were hemoglobin (g/dl), RBC count (million/L), RDW (%), MCV (fl) and MCH (pg).

The HPLC tests were performed on Variant II manufactured by BIO RAD laboratories, USA. Samples were stored at 4–8 °C and were analyzed in batches within 1 week. The instrument is based on chromatographic separation of analytes by ion exchange. Variant-II delivers a programmed buffer gradient of increasing ionic strength to the cartridge, where the hemoglobins are separated based on their ionic interactions with the cartridge material. The separated hemoglobins then pass through flow cell of the filter photometer, where changes in the absorbance at 415 nm are measured. Hb A2/F calibrator and two levels of controls (BIO-RAD) were analyzed at the beginning of each run.

Serum ferritin level was assessed by ARCHITECT ferritin assay which is a chemiluminescent microparticle immunoassay for the quantitative determination of ferritin in human serum and plasma [Ferritin kit-7K59].

The 12 discrimination indices used in the evaluation were calculated and are summarized in Table 1.

The cut off for discrimination index for mean density of hemoglobin per liter of blood (MDHL) and median of mean cell hemoglobin density (MCHD) was calculated by taking 60 random samples (30 males and 30 females) with normal Hb, MCV, MCH values and computing their mean using the Windows Microsoft Excel Software 2003.

Statistical analysis was done using Graph Pad prism software. P value of < 0.05 was considered to be significant. Sensitivity, specificity, Positive predictive value, Negative Predictive Value, Accuracy and Youden's Index of each index to detect BTT versus IDA was compared according to the formulas given below.

$$\text{Sensitivity} = [\text{True Positive}/(\text{True Positive} + \text{False Negative})] \times 100$$
$$\text{Specificity} = [\text{True Negative}/(\text{True Negative} + \text{False Positive})] \times 100$$
$$\text{Positive Predictive Value} = [\text{True Positive}/(\text{True Positive} + \text{False Positive})] \times 100$$
$$\text{Negative Predictive Value} = [\text{True Negative}/(\text{True Negative} + \text{False Negative})] \times 100$$
$$\text{Youden's Index} = [(\text{Sensitivity} + \text{Specificity}) - 100]$$

Table 1 Formula of each discrimination index

Formula	Equation	Cut off	
		BTT	IDA
Mentzer Index (MI)	MCV/RBC	≤ 13	> 13
Shine and Lal Index (SLI)	MCV × MCV × MCH/100	≤ 1530	> 1530
England and Fraser Index (EFI)	MCV − (5 × Hb) − RBC − 3.4	≤ 0	> 0
Green and King Index (GKI)	(MCV × MCV × RDW)/(Hb × 100)	≤ 65	> 65
Srivastav Index (SRI)	MCH/RBC	≤ 3.8	> 3.8
Ricerca Index (RI)	RDW/RBC	> 4.4	≤ 4.4
Sirdah (SI)	MCV − RBC − (3 × Hb)	≤ 27	> 27
Ehsani (EI)	MCV − (10 × RBC)	≤ 17	> 17
MDHL[a]	(MCH/MCV) × RBC	> 1.67	≤ 1.67
MCHD[b]	MCH/MCV	> 0.35	≤ 0.35
Red Cell Distribution Width Index (RDWI)	MCV × RDW/RBC	≤ 220	> 220
RBC count (in millions)	–	> 5.5	≤ 5.5

[a]MDHL > the mean MDHL of the normal population was evaluated as BTT and MDHL < the mean MDHL of the normal population was evaluated as IDA

[b]MCHD > the mean MCHD of the normal population signified BTT and MCHD < the mean MCHD of the normal population signified IDA

Results

Out of 1353 cases, 81.45% (1102/1353—Male:Female = 1:1.5) cases were diagnosed as Iron deficiency anemia. Confirmed cases of IDA were lost to follow up after initiating iron therapy and therefore could not be revaluated to rule out a masked thalassemic condition.

10.27% cases (139/1353—Male:Female = 1:1.5) displayed abnormal hemoglobin fraction on HPLC graph. Beta thalassemia trait formed the largest sub group representing 70.5% (98/139—Females: 61, Males: 37) of the total abnormal hemoglobin variants. There were 19 (13.7%) cases of beta thalassemia major and 15 (10.8%) cases of thalassemia intermedia. Rare variants included two cases each (1.4%) of Hb J Meerut and Hb Lepore. One case each (0.7%) of Hb D-Iran, Hb E-beta thalassemia trait double heterozygous and Hb E heterozygous case (0.7% of the total cases) were diagnosed.

Remaining 112 samples (8.28%) were cases with microcytic hypochromic morphology (Hb < 13gm/dl and MCV < 80 fl) but normal Hb A2 and HbF fractions on

Table 3 Mean value of the indices

Indices	BTT (N = 98)	IDA (N = 1102)	*P* value
RI	3.1 ± 0.5	5.4 ± 1	< 0.0001
GKI	59 ± 17.6	117.2 ± 37.6	< 0.0001
MI	12.8 ± 1.8	17.6 ± 3.2	< 0.0001
EI	13.8 ± 7.3	29.5 ± 10	< 0.0001
RDWI	203.3 ± 37.3	381 ± 80.4	< 0.0001
EFI	− 2.0 ± 9.9	14.9 ± 10.6	< 0.0001
SI	24.8 ± 7.7	37.5 ± 8.4	< 0.0001
RBCC	5.1 ± 0.6	4.1 ± 0.6	< 0.0001
MDHL	1.7 ± 0.4	1.4 ± 0.4	< 0.0001
SRI	4.2 ± 0.6	6.1 ± 1.3	< 0.0001
SLI	908 ± 152	1196.5 ± 236.8	< 0.0001
MCHD	0.3 ± 0.1	0.3 ± 0.1	1.0

Table 2 CBC parameters among the cases of BTT and IDA

Parameters	BTT (N = 98) Mean ± SD	IDA (N = 1102) Mean ± SD	Co-existent BTT and IDA (N = 29) Mean ± SD
Hemoglobin (gm/dl)	12.1 ± 0.4	9.6 ± 2	9.1 ± 0.3
RBC (million)	5.1 ± 0.6	4.1 ± 0.6	4.8 ± 0.2
MCV (fl)	65 ± 4.6	70.3 ± 7	62.9 ± 1.7
MCH (pg)	24.2 ± 3.4	26.9 ± 3.4	20.6 ± 1.0
RDW (%)	15.9 ± 2.3	21.7 ± 3.3	19.5 ± 2.5

HPLC and normal or increased Serum Ferritin levels. These cases could have been of Anemia of chronic disorders but were not analyzed further.

Mean values of the hematological parameters for IDA and BTT cases in our study are shown in Table 2. BTT cases had a mean Hb value of 12.1 ± 0.4 g/dl and IDA cases had a mean Hb concentration of 9.6 ± 2 g/dl. The mean value of MCV was 65 ± 4.6 fl in the BTT category which was lower when compared to the mean value of 70.3 ± 7 fl in IDA cases. The mean value of RDW and RBC in BTT group was 15.9 ± 2.3% and 5.1 ± 0.6 million respectively and in IDA group was 21.7 ± 3.3% and 4.1 ± 0.6 million respectively, and both were found to be statistically significant (p value < 0.0001). The mean value of the cut off all the indices in the BTT and IDA cases are summarized in Table 3. The values were found to be statistically significant (P value < 0.0001),

A marked variation was seen in the number of correctly diagnosed cases between the two categories as evident from Table 4. In case of BTT, Ricerca Index and Shine and

Table 4 Diagnostic accuracy of discrimination indices

Index	Correctly diagnosed cases	Sensitivity	Specificity	PPV	NPV	Accuracy	Youden's Index
RI							
BTT	98 (100%)	100	87.39	41.35	100	88.42	87.39
IDA	963 (87%)	87.39	100	100	41.35		
GKI							
BTT	77 (79%)	78.57	94.01	53.85	98.01	92.75	72.58
IDA	1036 (94%)	94.01	78.57	98.01	53.85		
MI							
BTT	79 (81%)	80.61	88.75	38.92	98.09	88.08	69.36
IDA	978 (89%)	88.75	80.61	98.09	38.92		
EI							
BTT	79 (81%)	80.61	88.75	38.92	98.09	88.08	69.36
IDA	978 (89%)	88.75	80.61	98.09	38.92		
RDWI							
BTT	69 (70%)	70.41	98.37	73.31	97.39	96.08	68.78
IDA	1084 (98%)	98.97	70.41	97.39	73.31		
EFI							
BTT	76 (78%)	77.55	91.02	43.43	97.85	89.92	68.57
IDA	1003 (91%)	91.02	77.55	97.85	43.43		
SI							
BTT	76 (81%)	77.55	87.39	35.35	97.77	86.58	64.94
IDA	963 (87%)	87.39	77.55	97.77	35.35		
RBCC							
BTT	62 (63%)	63.27	91.83	40.79	71.43	96.56	55.1
IDA	1012 (92%)	91.83	63.27	71.43	40.79		
MDHL							
BTT	61 (62%)	62.24	78.58	20.54	95.90	77.25	40.82
IDA	866 (79%)	78.58	62.24	95.90	20.54		
SRI							
BTT	34(35%)	34.69	97.37	53.9	94.37	92.25	32.06
IDA	1073 (97%)	97.97	34.69	94.37	53.9		
SLI							
BTT	98 (100%)	100	3.54	8.44	100	11.42	3.54
IDA	39 (4%)	3.54	100	100	8.44		
MCHD							
BTT	40 (41%)	40.82	55.81	7.59	91.38	54.58	− 3.37
IDA	615 (56%)	55.81	40.82	91.38	7.59		

Lal correctly diagnosed all the cases in BTT (100%) while Srivastav Index could diagnose the least number of cases (35%). In IDA, RDWI was found to be most accurate, correctly diagnosing 98% of the cases followed by Srivastav Index (97%).

There were 29 cases diagnosed with co-existent BTT and IDA. Indices cannot be used for screening in these cases because of different patho-physiology. BTT leads to an increased RBC count with a near normal RDW while IDA yields a low RBC count and a high RDW. The mean values of RBC and RDW in these coexistent cases in this study were 4.8 ± 0.2 million and $19.5 \pm 2.5\%$ respectively (Table 3). Based on this analysis a discrepancy in the hematological parameters showing an increase in both RBC count and RDW may be suspected with a coexistent condition. However, evaluation of indices in such cases will give an inconclusive result. Ricerca Index in these cases could correctly diagnose only 24% of these cases while it shows 100% results in pure BTT cases.

Sensitivity, specificity, positive and negative predictive values and Youden's Index of the discrimination indices is shown in Table 4.

Ricerca Index and Shine and Lal Index, both showed highest sensitivity of 100% in detecting BTT cases. Mentzer Index and Ehsani Index both had a sensitivity of 80.61% each, which was followed by Green and King, England and Fraser, Sirdah Index and RDWI.

RDWI showed highest specificity of 98.37% in detecting BTT which was closely followed by Srivastav and Green and King Index. Moreover, RDWI also showed a highest PPV.

For the detection of IDA, highest sensitivity of 98% was seen for RDWI which was closely followed by Srivastav Index, Green and King Index and RBCC with their sensitivity ranged from 91 to 98%. Ricerca and Shine and Lal Index had a specificity and PPV of 100% each.

Based on Youden's Index which measures the diagnostic test's ability to balance sensitivity and specificity, the indices in the decreasing order of their efficacy in our study is as follows: RI > GKI > MI > EI > RDWI > EFI > SIR > RBCC > MDHL > SRI > SLI > MCHD.

Discussion

Subtle variations were seen in the RBC parameters obtained in CBC in cases of BTT and IDA according to our data. BTT group showed a higher degree of microcytosis and hypochromia with lesser variation in red cell size while IDA group showed significant variation in its RDW. The mean values of the hematological parameters in our study was concordant with the findings of Sirdah et al. [2], Bhushan et al. [3], Kumar et al. [4], Meshram et al. [5], Vehapoglu et al. [6] and Chuan et al. [7].

RDWI correctly diagnosed a highest number of cases (96%) of IDA and BTT in the present study. Green and King was the second best index correctly diagnosing 93% of the cases. This finding was comparable with the study of Bhushan et al. [3] with 97% cases being correctly diagnosed by RDWI. A slightly lower but similar result for RDWI was observed by Vehapoglu et al. [6] and Niazi et al. [8] with number of correctly diagnosed cases of 80% and 88% respectively. Other indices evaluated in the present study were analogous with the results of Bhushan et al. [3] and Vehapoglu et al. [6] while the other studies showed variations in their results.

Ricerca Index and Shine and Lal Index were found to have the highest sensitivity in our study (100%) for differentiating BTT from iron deficiency anemia. This finding was similar to the Indian studies done by Singh et al. [9], Rathod et al. [10], Bhushan et al. [3], Meshram et al. [5] and Mukhopadhyay et al. [11]. Our findings were also concordant with studies by Vehapoglu et al. [6], Demir et al. [12], Chuan et al. [7] and Okan et al. [13]. A lower sensitivity for Shine and Lal Index was seen in studies by Sirdhah et al. [2] and Niazi et al. [8] while Suad et al. [14] reported a lower sensitivity for Ricerca Index.

In the present study RDWI showed a highest specificity of 98.37% which was closely followed by Srivastav Index, Green and King Index and England and Fraser Index with a specificity of 97.37%, 94% and 91.02% respectively. Our results for RDWI was concordant to the studies done by Bhushan et al. [3], Ntaois et al. [15], Okan et al. [13] and Chuan et al. [7].

The least specific Index for detecting BTT cases based on our study was Shine and Lal with 3.5% specificity. Other studies have also observed similar lower specificity for SLI ranging from 0 to 10% [3, 5–7, 12, 14] However, a higher specificity of 97% and 100% has also been demonstrated by Okan et al. [13] and Niazi et al. [8] respectively.

While few indices showed a sensitivity of 100%, their specificity was low which meant more number of false positive cases. Youden's Index (YI) measures a diagnostic test's ability to balance sensitivity and specificity. Therefore, based on Youden's Index, the best three indices in the decreasing order of their efficacy in our study were Ricerca Index > Green and King Index and Mentzer Index as evident from Table 4.

For Ricerca Index, Meshram et al. [5] observed a slightly higher but similar Youden's Index while majority of the studies showed a lower value ranging between 50 and 70% [2, 7, 8, 13, 14]. YI as low as 14.7% and 29.6% was also observed by Vehapoglu et al. [6] and Rathod et al. [10] respectively.

Green and King Index showed results of YI comparable to Sirdah et al. [2], Okan et al. [13], Ntaois et al. [15], Chuan et al. [7] and Demir et al. [12]. A lower value ranging from 30 to 60% was observed by the other studies [3, 5, 11, 14].

For MI, Niazi et al. [8], Okan et al. [13], Suad et al. [14] and Sirdah et al. [2] observed results similar to the present study. A higher Youden's Index of 92%, 90.1% and 8% was observed by Rathod et al. [10], Ehsani et al. [16] and Vehapoglu et al. [6] respectively; while the other studies demonstrated a lower value ranging between 48 and 58% [5, 7, 11, 12, 15].

Thus the present study reflects the magnitude of thalassemia and other hemoglobinopathies in a small hospital based population. This may very well represent the tip of an iceberg, as the burden of disease is of much higher magnitude. We propose screening of all the microcytic hypochromic cases of anemia using a reliable index followed by confirmatory tests using HPLC as an ideal method for the preliminary diagnosis of hemoglobinopathies. Ricerca and Green and King Index were found to have a better diagnostic accuracy compared to the commonly used Mentzers Index. Use of these indices for population screening, genetic counseling and prenatal diagnosis can help to effectively counter the enormity of the problem.

Compliance with Ethical Standards

Conflict of interest None.

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