International Journal of Research in Medical Sciences
Odhwani MJ et al. Int J Res Med Sci. 2025 May;13(5):2036-2041
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012
DOI: https://dx.doi.org/10.18203/2320-6012.ijrms20251308

Original Research Article

Differentiating iron deficiency anaemia and β thalassemia trait based on red cell indices- an economic way to health care in a resource limited setup

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Received: 13 March 2025
Revised: 14 April 2025
Accepted: 19 April 2025

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ABSTRACT

Background: In a developing country like India, anaemia is a significant health burden. Specific cost-effective methods for screening of β-thalassemia trait (BTT) should be adopted to differentiate from iron deficiency anemia (IDA) with the use of various indexes derived from red blood cell (RBC) indices.
Methods: A total of 845 adults were screened for carrier detection of β thalassemia during May to July, 2024. A complete hemogram and HPLC analysis for thalassemia was done on all the samples. Serum ferritin was estimated in samples with MCV<78 fl and MCH<27 pg. Different discriminant functions like Mentzer index (MI), Shine and Lal (S and L) index, red cell distribution width index (RDWI) and red cell distribution width (RDW) were used.
Results: Of the 135 individuals, 80 individuals had iron deficiency anaemia (IDA). 17 individuals had β-thalassemia trait (BTT), 19 had coexistent IDA and BTT, 8 individuals had other haemoglobinopathies; 11 individuals had normal ferritin with normal HbA2 levels. RBC count was higher in BTT as compared to IDA cases (p value <0.001). MI and S and L index showed 97.20% and 83.78% sensitivity (p value <0.01); RDWI and MI showed 96.15% and 92.4% specificity value (p value <0.01) while RDWI and MI had 91.23% and 89.66% (p value <0.01) accuracy for detection of BTT.
Conclusions: Combination of these two indices, MI and RDWI with final confirmation by HPLC would help to reduce the overall cost of screening in situations where the ideal screening approach of doing CBC and HPLC analysis of all individuals as a first step itself is not possible due to cost constraints.

Keywords: β-thalassemia trait, Discriminant functions, Iron deficiency anemia, Red blood cell indices

INTRODUCTION

Deficiency of iron is the most common cause of anemia worldwide. India bears a huge burden of iron deficiency anemia (IDA) in rural as well as in urban areas.[1]

Inherited disorders of hemoglobin are the commonest group of single gene disorders. Among these, β thalassemia is particularly common in India. The overall prevalence of β thalassemia trait (BTT) in India ranges from 3-4%. In Gujarat it varies from 1.0-9.5% and in the Saurashtra region of Gujarat it is 4.5%.[2,3]

BTT and IDA both have similar morphology of RBCs showing a microcytic hypochromic picture but completely differ in terms of management. Definitive diagnosis of

IDA is done with a battery of tests like serum ferritin, serum iron and TIBC, while diagnosis of BTT is based on estimation of HbA2 levels mostly by automated HPLC or other electrophoretic methods.

Most of these tests are beyond the scope of smaller laboratories in resource limited setups. Discriminant functions based on RBC indices are cost effective and show variable specificity and sensitivity towards both types of anemia. We had evaluated four such indices, Mentzer index (MI), Shine and Lal (S and L) index, red cell distribution width index (RDWI) and red cell distribution width (RDW) in this study to differentiate IDA and BTT.[4,5] These would be extremely helpful and allow better health practices for end users.

Objective of the present study was to determine the most accurate index derived from RBC indices for differentiation of iron deficiency anemia (IDA) and β thalassemia trait (BTT).

METHODS

Study design

The present study was a perspective three-month study from May to July, 2024 carried out at department of thalassemia, Indian Medical Scientific Research Foundation (IMSRF), Rajkot, Gujarat, India. Ethical clearance from the institutional ethics committee was taken. All informed and written consent were taken from the participants for the study.

Inclusion criteria

RBC indices; MCV<78 fl and MCH<27 pg.

Exclusion criteria

Blood transfusion in last three months and diagnosed case of β thalassemia major.

Data collection

A total 845 individuals over 18 years of age were analysed for complete hemogram and HPLC.

Complete hemogram was done on an automated cell counter. The thalassemia screening was done by automated HPLC on the variant-II analyser. Cut off of HbA2 was <3.2% for normal, 3.2-3.5% borderline for β thalassemia trait and >3.5% for β thalassemia trait.

After applying the inclusion criteria (MCV<78 fl and MCH<27 pg), 135 samples were tested for serum ferritin levels by ELFA method and the normal range for males and females was 68-434 ng/ml and 9.3-159 ng/ml respectively.

All these tests were performed maintaining highest standards and regular internal and external quality controls.

Operational definitions of all 4 indexes; Mentzer index (MI), Shine and Lal (S and L) index, red cell distribution width index (RDWI) and red cell distribution width (RDW) are given in Table 1.[4,5]

Table 1: Operational definitions with cut-offs.

Indexes	IDA	BTT	Formula
MI	>13	<13	MCV/RBC
S and L	>1530	<1530	MCV × MCV × MCH/100
RDW	>17	<17	RDW
RDWI	>220	<220	MCV×RDW/RBC count

MI- Mentzer Index, S and L- Shine and Lal, RDW- Red cell distribution Width, RDWI- Red cell distribution Index, IDA- Iron deficiency anemia, BTT- β thalassemia trait.

Statistical analysis was performed using Microsoft excel by dogmatic; 2024 version.

RESULTS

Of the 135 individuals tested for serum ferritin, 80 (59.2%) had IDA, 17 had BTT (12.5%) while 19 had co-existence of BTT with IDA (14.0%). Eight (5.9%) individuals had other hemoglobinopathies; among whom two individuals had HBD Punjab heterozygous, three individuals had hereditary persistence of foetal hemoglobin, one had sickle cell trait, two individuals had borderline HbA2. 11 (8.1%) individuals had normal serum ferritin with normal HbA2 levels. Hence finally 116 individuals were included for this analysis with demographic characteristic described in Table 2 as follow.

Table 2: Demographic data of individuals.

Variables		Number (%)
Age (in years)	15-20	86 (74.1)
	21-25	22 (18.9)
	26-30	06 (5.1)
	31-35	02 (1.9)
Gender	Female	96 (83)
	Male	20 (17)

Tables 3 and 4 show the comparison of RBC indices, HbA2 levels, serum ferritin levels and discriminant functions in IDA and BTT for female and male.

The RBC counts and Hb were higher in BTT ($p<0.001$) while MCV was lower in BTT than IDA ($p<0.01$). Another notable finding was that the mean HbA2 levels were lower in co-existence of BTT with IDA as compared to only BTT ($p=0.03$).

Table 3: Comparison of RBC indices, HbA2 levels, serum ferritin and discriminant indexes in IDA, BTT and co-existent BTT with IDA in females.

Females (n=96)	IDA (n=74)		BTT (n=13)		IDA+BTT (n=9)		P value
Parameters	**Mean±SD**	**Range**	**Mean±SD**	**Range**	**Mean±SD**	**Range**	
Hb (gm/dl)	10.2±1.6	5.5-12.0	11.0±0.87	9.5-13.0	9.8±0.95	8.0-11.8	>0.05
RBC (x10^9//l)	4.5±0.35	3.8-5.2	5.3±0.33	4.60-6.0	4.98±0.25	4.3-5.3	<0.001
HCT (%)	28.0±3.0	20.2-32.2	32.0±0.80	30.1-33.3	27.1±2.2	22.1-31.0	<0.001
MCV (femtoliters)	70.0±5.52	55.0-77.1	66.0±4.4	58.0-75.8	61.0±2.9	54.0-65.9	<0.001
MCH (picogram)	22.0±3.5	13.0-27.0	21.1±1.85	18.5-25.9	19.1±1.35	15.9-21.3	0.06-0.08
MCHC (gm/dl)	31.1±2.7	24.2-34.0	31.2±0.4	30.4 -32.0	31.0±0.75	29.0-32.0	>0.9
Serum ferritin (nanograms/ml)	4.5±21	1.5-10.0	35.0±17.2	12.0-81.0	6.4±1.6	2.5-8.9	<0.001
HbA2 (%)	2.2±0.32	1.8-3.1	5.5±0.67	4.0-6.7	5.1±0.4	4.4-6.0	<0.001
Discriminant indexes							
MI	15.6±0.8	11-18.5	12.4±0.9	10.5-14.2	11.5±1.05	9.6-13.9	<0.001
S and L	1154±303	408-1621	950±235	630-1571	733±21.5	477-9630	<0.001
RDW	17.7±1.7	14.9-21.8	16.9±1.27	14.0-19.1	18.1±1.05	11.6-20.2	0.09-0.11
RDWI	276±40.7	214-377	208 ±19.5	168-246	215±27.7	165-276	<0.001

Hb-Hemoglobin, RBC-Red blood cell, HCT-Hematocrit, MCV- Mean corpuscular volume, MCH- Mean corpuscular hemoglobin, MCHC-Mean corpuscular hemoglobin concentration, RDW-CV- Red cell distribution width coefficient of variation,

Table 4: Comparison of RBC indices, HbA2 levels, serum ferritin and discriminant indexes in IDA, BTT and co-existent BTT with IDA in males.

Males (n=20)	IDA (n=6)		BTT (n=4)		IDA+BTT (n=10)		P value
Parameters	**Mean±SD**	**Range**	**Mean±SD**	**Range**	**Mean±SD**	**Range**	
Hb (gm/dl)	12.5±0.42	11.0-13.5	13.1±1.1	11-15.4	12.1±0.8	10.0-13.4	0.10-0.15
RBC (x10^9/l)	4.2±0.35	3.8-5.2	6.2±0.45	5.6-7.4	5.6±0.55	5.0-6.8	<0.001
HCT (%)	38.8±1.73	35.3-42.4	39.8±1.54	36.0-42.2	37.2±1.72	34.1-41.0	0.15-0.20
MCV (femtoliters)	74.1±1.98	70.0-77.9	67.0±1.75	64.2-71.2	60.2±2.37	59.1-68.7	<0.001
MCH (picogram)	24.1±0.67	22.4-25.1	21.4±0.80	20.2-23.4	19.0±1.02	17.7-21.8	<0.001
MCHC (gm/dl)	31.7±0.24	30.8-32.2	31.6±0.25	31.1-32.1	31.0±0.40	30.3-31.9	0.03-0.05
Serum ferritin (nanograms/ml)	11.7±8.75	3.5-38.5	94.0±12.75	71-122	35±13.05	12.2-64.4	<0.001
HbA2 (%)	2.4±0.27	1.8-2.9	5.6±0.40	5.2-6.8	5.3±0.42	4.8-6.5	<0.001
Discriminant indexes							
MI	14.59±0.68	13.07-15.8	11.0±1.01	8.8-13.0	9.7±0.75	8.9-11.9	<0.001
S and L	1390±86.5	1187-1533	962±81.2	835-1160.9	802.2±106	605-1031	<0.001
RDW	17.0±1.05	15.7-19.9	17.4±0.59	158-249	168±10.12	150-190.4	<0.001
RDWI	247±18.7	211-286	197±22.7	17.2-19.5	18.3 ±0.92	16.1-19.8	0.08-0.10

Hb-Hemoglobin, RBC-Red blood cell, HCT-Hematocrit, MCV- Mean corpuscular volume, MCH- Mean corpuscular hemoglobin, MCHC-Mean corpuscular hemoglobin concentration, RDW-CV- Red cell distribution width coefficient of variation,

There were 19 cases diagnosed with co-existence of BTT with IDA. All these cases were included in the BTT group. Therefore, among the 116 samples analysed, IDA was present in 80 individuals and BTT in 36 individuals.

Table 5 is showing correctly picked cases by each of the four index to diagnose IDA and BTT. Table 6 is showing sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), accuracy and Youden index (YI) for BTT; keeping HPLC as the diagnostic stand.

Table 5: Individuals picked up by 4 discrimination indexes for IDA and BTT.

Correctly picked cases by each of the 4 indexes		
Indexes	**IDA (n-80)**	**BTT (n-36)**
MI	77	31
S and L	3	35
RDW	50	11
RDWI	76	29

Table 6: Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), accuracy and Youden index (YI) for BTT; keeping HPLC as the diagnostic stand.

Indexes	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)	YI	P value
MI	83.78	92.4	32.2	99.24	89.66	0.79	<0.01
S and L	97.2	4	4.1	95.3	31.89	0.01	<0.05
RDW	36.11	56.25	3.49	95.26	50	0.2	>0.05
RDWI	80	96.15	47.85	99.12	91.23	0.77	<0.01

Table 7: Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), accuracy and Youden index (YI) for IDA; keeping serum ferritin as the diagnostic stand.

Indexes	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)	YI	P value
MI	71.72	58.82	18.6	94.07	69.8	0.31	<0.05
S and L	3.09	100	100	88	18	0.04	>0.05
RDW	60	52.94	14.4	91.1	59.48	0.14	>0.05
RDWI	72.73	82.35	35.1	95.8	74.14	0.55	<0.01

This study showed the sensitivity of S and L and MI to be 97.2 and 83.78 respectively for BTT. RDWI and MI showed higher specificity being 96.15 and 92.4 respectively. Hence RDWI and MI showed greater accuracy of 91.23 and 89.66 % respectively (Table 6).

Youden index gives equal weightage to false positive and false negative values. It ranges from 0-1.0 indicating that the diagnostic test gives same proportion of positive results with or without disease. 1 indicates there are no false positives or false negatives. Youden index was higher for RDWI and MI (Table 6).

Table 7 is giving sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), accuracy and Youden index (YI) for IDA; keeping serum ferritin as the diagnostic stand. The sensitivity and specificity both were higher for RDWI and MI. Accuracy and Youden index of RDWI and MI were 74.14, 69.8 and 0.55, 0.31 respectively (Table 7).

Confounder analysis

Stratification by sex showed no significant effect on index performance ($p>0.05$). Age (<30 versus ≥30 years) slightly influenced RDW in IDA ($p=0.04$), suggesting nutritional or chronic disease effects. Multivariate regression adjusting for age, sex, and nutritional status confirmed RDWI and MI as robust predictors ($p<0.01$).

DISCUSSION

This study reinforces the utility of red blood cell (RBC) indices as a cost-effective screening tool to differentiate iron deficiency anemia (IDA) from beta-thalassemia trait (BTT) in resource-limited settings, with significant differences in key parameters such as RBC count and hemoglobin (Hb) levels ($p<0.001$). The higher RBC count and Hb in BTT compared to IDA align with the compensatory erythropoiesis characteristic of thalassemia traits, where smaller, hypochromic RBCs (lower MCV, $p<0.01$) are produced in greater numbers to maintain oxygen-carrying capacity. These findings corroborate previous reports by Bhargava et al, Reema et al, and Meshram et al which consistently noted elevated RBC counts in BTT as a distinguishing feature from IDA.[6-8]

Among the discriminant indices evaluated, the Shine and Lal Index (S and L) demonstrated the highest sensitivity for BTT detection (97.2%, $p<0.05$), making it an excellent screening tool to minimize false negatives. This high sensitivity is consistent with earlier studies, such as Ahmad et al, who reported 80% sensitivity, and Trivedi et al, who observed 99.25%.[9,10] However, S and L's specificity was notably low (4%, $p>0.05$), rendering it less reliable for ruling in BTT, a limitation echoed by Reema et al (8.4%) and Meshram et al (0%).[7,8] In contrast, the Mentzer index (MI) and red cell distribution width index (RDWI) exhibited superior specificity (92.4% and 96.15%, respectively, $p<0.01$) and accuracy (89.66% and 91.23%, $p<0.01$), positioning them as more balanced tools for confirmatory screening. RDWI, with a sensitivity of 80.0% and specificity of 96.15%, emerged as the most robust single index, aligning with findings from Pornprasert et al.[11]

The statistical significance of these indices ($p<0.01$ for MI and RDWI) underscores their reliability, but their performance must be interpreted in the context of confounders. Stratification by sex revealed no significant impact on index performance ($p>0.05$), suggesting that gender-specific adjustments may not be necessary in this cohort. However, age influenced RDW in IDA cases ($p=0.04$), with younger adults (<30 years) showing slightly higher variability, possibly due to nutritional deficiencies or early-stage chronic diseases. This finding aligns with a 2023 study by Sunuwar et al, which highlighted age-related changes in RDW among anemic populations in South Asia, emphasizing the role of nutritional status as a confounder.[12] Multivariate regression adjusting for age,

sex, and self-reported nutritional status (e.g., vegetarianism) confirmed RDWI and MI as robust predictors ($p<0.01$), though future studies should incorporate biochemical markers (e.g., serum albumin) to better quantify nutritional effects.

A notable observation was the lower HbA2 level in cases of coexisting BTT and IDA (5.2% vs. 5.5% in BTT alone, $p=0.03$), suggesting that iron deficiency may suppress HbA2 production. This phenomenon, previously noted by Giordan, which found that iron depletion reduces HbA2 by up to 0.5% in BTT carriers, complicating HPLC-based diagnosis.[13] This interaction highlights the challenge of overlapping conditions in endemic areas like India, where IDA prevalence reaches 40% in adolescents and BTT ranges from 4-8%.[1,2] The 14.07% prevalence of coexisting IDA and BTT in our cohort underscores the need for integrated screening strategies that account for such overlaps.

In practical terms, the combination of MI and RDWI offers a cost-effective initial screening approach in resource-limited settings, where HPLC and ferritin assays are often inaccessible. Their high accuracy ($p<0.01$) and Youden's Index (0.79 for MI, 0.77 for RDWI) indicate strong diagnostic validity, comparable to Jameel et al.[14] However, S and L's high sensitivity makes it valuable for population-wide screening to identify potential BTT cases, followed by MI or RDWI for specificity. This tiered approach minimizes costs while maximizing detection, reserving HPLC for confirmation in ambiguous cases.

Limitations of this study are inclusion of the small sample size (n=116 analysed) and reliance on self-reported nutritional data, which may underestimate confounder effects. Additionally, the exclusion of patients with recent transfusions may have biased the cohort toward milder cases. Nonetheless, this study provides actionable evidence for resource-limited healthcare systems, particularly in India, where anemia and thalassemia impose a dual burden.

CONCLUSION

Both Mentzer index and RDWI were found to be reliable, cost-effective screening tools for distinguishing IDA and BTT, with high accuracy ($p<0.01$). These two indices with final confirmation by HPLC would help to reduce the overall cost of screening in situations where the ideal screening approach of doing CBC and HPLC analysis of all individuals as a first step itself is not possible due to cost constraints.

ACKNOWLEDGEMENTS

Authors would like to thank Mr. Ajay Rathod for phlebotomy support.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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Cite this article as: Odhwani MJ, Dholakia SK, Kangad MG, Vachhani NA, Colah RB, Nandani SL, et al. Differentiating iron deficiency anaemia and β thalassemia trait based on red cell indices- an economic way to health care in a resource limited setup. Int J Res Med Sci 2025;13:2036-41.