

## Research Paper

## Diagnostic Value of Red Blood Cell Indices in Differentiating Iron Deficiency Anemia From Beta Thalassemia Trait

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## ABSTRACT

**Background:** Iron deficiency anemia (IDA) and beta thalassemia trait (BTT) are the most common forms of microcytic hypochromic anemia in pediatric populations.

**Objectives:** This study aimed to assess and compare the diagnostic performance of six red blood cell (RBC) indices in distinguishing BTT from IDA.

**Methods:** In this cross-sectional study, 148 pediatric patients diagnosed with either BTT or IDA were enrolled. RBC indices were measured in both groups, and various diagnostic formulas were applied. Statistical analysis was performed using SPSS software, version 21.

**Results:** Among the 148 patients (aged 6 months to 18 years), 74 had IDA and 74 had BTT. The RBC count index demonstrated the highest diagnostic accuracy (83.87%) in differentiating the two conditions, with a Youden index of 67%. After adjusting diagnostic thresholds, RBC count remained the most effective indicator (Youden index: 71.6%), followed by the RDWI, Mentzer, England & Fraser, Sirvastava, and Shin & Lal indices.

**Conclusions:** The RBC count index showed the highest diagnostic value among all evaluated indices and may serve as a reliable, accessible tool for differentiating IDA from BTT in clinical settings.

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## Introduction

Iron deficiency anemia (IDA) and beta thalassemia trait (BTT) are among the most common causes of microcytic hypochromic anemia in children [1]. BTT is an autosomal recessive disorder characterized by impaired synthesis of beta-globin chains [2], while IDA is a widespread nutritional deficiency associated with anemia, developmental delays, and behavioral disturbances [3].

Beta-thalassemia is classified into three clinical types based on severity: Beta-thalassemia minor, intermedia, and major. BTT typically presents with reduced mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH), along with elevated hemoglobin A2 (Hb A2) levels and increased red blood cell (RBC) count [2]. However, differentiating BTT from other microcytic anemias using conventional laboratory parameters remains challenging. Although BTT is often asymptomatic and does not require treatment, its early identification is crucial for genetic counseling—particularly in regions where consanguineous marriages are prevalent—to prevent the birth of children with beta-thalassemia major. Diagnosis is generally based on hematologic indices, including MCV, MCH, and red cell distribution width (RDW), but these parameters may overlap significantly with those seen in IDA [4, 5].

Inadequate differentiation between BTT and IDA may result in misdiagnosis, leading to inappropriate treatment with iron supplements in patients with BTT. This not only fails to resolve the anemia but may also cause iron overload and related complications [5]. While confirmatory tests—such as serum ferritin measurement, Hb electrophoresis (to determine Hb A2 levels), and DNA analysis—are available, they are costly, time-consuming, and may not be accessible in low-resource settings, especially rural areas. To overcome these limitations, several cost-effective differential diagnostic indices have been proposed, utilizing simple RBC-derived parameters such as MCV, MCH, RDW, and RBC count, which are readily obtained from automated blood analyzers [5].

Iron deficiency remains the most prevalent nutritional deficiency in childhood. A global estimate from 2010 reported a 32.9% prevalence of anemia, with the highest burden observed in children under five years of age [6]. Thalassemia, a highly prevalent hereditary hemoglobinopathy, is endemic in the Eastern Mediterranean region [7]. In Iran, the prevalence of BTT is high,

ranging from 4% to 8% nationwide, and reaching up to 10% in the southeastern province of Sistan-Baluchestan. In this province alone, more than 3,000 registered patients with thalassemia major require regular blood transfusions [2].

Given the high prevalence of both IDA and BTT in Sistan-Baluchestan Province, along with the common practice of consanguineous marriage, accurate diagnosis of BTT is essential to reduce the incidence of thalassemia major. Misclassification of these two conditions can have serious health and societal consequences. Therefore, there is a pressing need for rapid, affordable, and reliable diagnostic tools to distinguish between IDA and BTT, especially in underserved regions [8–10].

Considering the high disease burden and limited access to advanced diagnostic modalities, simple hematological indices play a pivotal role in the differential diagnosis of BTT and IDA. This study aimed to evaluate and compare the diagnostic utility of six indices—Shin & Lal, Sirvastava, Mentzer, England & Fraser, RDWI, and RBC count—by calculating their sensitivity, specificity, positive predictive value (PPV), negative predictive value, (NPV) and Youden index.

## Methods

This cross-sectional study was conducted among children aged 6 months to 18 years who were diagnosed with either beta-thalassemia trait (BTT) or IDA at the Hematology Clinic of Ali Asghar Children's Hospital in Zahedan, Iran. Patients were eligible for inclusion if they had a confirmed diagnosis of either BTT or IDA. Those with coexisting BTT and IDA, hemoglobin levels below 8 g/dL, other forms of anemia, or any acute or chronic inflammatory diseases, infections, hypothyroidism, malignancies, or recent significant bleeding were excluded.

The sample size was calculated using the Equation 1:

$$1. \quad x = \frac{z_{1-\alpha/2} \times SN(1-SN)}{L2 \times prevalence} = \frac{1.96 \times 0.95 \times 0.05}{(0.03)2 \times 0.7} = 148$$

(n=148), where Z was set at 1.96 for a 95% confidence interval, sensitivity (SN) at 0.95 as reported in previous studies [11, 12], an error margin (L) of 0.03, and a disease prevalence of 0.7. Based on this calculation, a sample size of 148 was obtained, with 74 patients assigned to the IDA group and 74 to the BTT group.

Demographic and clinical information, including age, sex, and complete blood count (CBC) parameters—such as Hb, RBC count, MCV, MCH, and red cell RDW—were retrieved from patient records. Additional laboratory findings included serum ferritin levels, hemoglobin electrophoresis results, and Hb A2 percentages. BTT diagnosis was confirmed through hemoglobin electrophoresis using the capillary zone method, demonstrating Hb A2 levels above 3.4%, along with hypochromic microcytosis in accordance with age- and sex-adjusted RBC indices, as measured by the Sysmex KX-21M hematology analyzer (Sysmex Corporation, Japan). IDA was diagnosed based on microcytic anemia, ferritin levels below 12 ng/mL in children under five and below 15 ng/mL in those over five, and the absence of inflammation or fever. Ferritin levels were measured using the enzyme linked immunosorbent assay (ELISA) method.

Following confirmation of diagnoses, RBC indices were evaluated, and the following formulas were used to differentiate between IDA and BTT: The Mentzer index (MCV/RBC), where values  $>13$  suggest IDA and  $<13$  suggest BTT; the Shin & Lal index  $[(MCV^2 \times MCH) \times 0.01]$  [14], with IDA  $>1530$  and BTT  $<1530$ ; the Sirvastava index (MCH/RBC), with IDA  $>3.8$  and BTT  $<3.8$ ; the England & Fraser index  $(MCV - RBC - 5 \times Hb - 3.4)$  [15, 16], with IDA  $>0$  and BTT  $<0$ ; and the red cell RDW index (RDWI), calculated as  $(MCV \times RDW)/RBC$ , where IDA  $>220$  and BTT  $<220$  [11, 14, 17].

Descriptive statistics, including Mean $\pm$ SD, were computed. The means of the variables between the two groups were compared using the independent t-test. For each diagnostic index, sensitivity, specificity, PPV, NPV, and the Youden index (calculated as sensitivity + specificity – 100) were assessed. True-positive, true-negative, false-positive, and false-negative values were also determined. All analyses were conducted using SPSS software, version 21.0 (IBM Corp., Armonk, NY, USA).

## Results

A total of 148 pediatric patients, aged between 6 months and 18 years, were included in the study. The cohort consisted of 77 boys and 71 girls. Of these, 74 were diagnosed with IDA and 74 with BTT. Ten RBC indices from the complete blood count (CBC) panel were evaluated and compared between the two groups. The summary of these hematological parameters is presented in Table 1.

## Determining the value of differential indices for accurate diagnosis

To assess the diagnostic performance of each index, the accuracy of six RBC-based formulas in distinguishing between IDA and BTT was calculated. Among these, the RBC count demonstrated the highest diagnostic accuracy at 83.87%, making it the most effective index for differential diagnosis. This was followed by the RDWI, Mentzer index, Sirvastava index, England & Fraser index, and Shin & Lal index, in descending order of diagnostic performance. The detailed accuracy values for each index are presented in Table 2.

Assessment of sensitivity, specificity, PPV, NPV, and the Youden index for each diagnostic index in differentiating IDA from BTT revealed that the RBC count index had the highest Youden index at 67%. This was followed by RDWI, Mentzer, Sirvastava, England & Fraser, and Shin & Lal indices, in descending order of diagnostic performance (Table 3; Figure 1).

To determine the optimal sensitivity and specificity of each differential index, receiver operating characteristic (ROC) curve analysis was performed across the entire study population. The diagnostic threshold for each index was selected based on the highest corresponding Youden index (Table 3).

After adjusting the diagnostic thresholds to achieve the highest Youden index, the RBC count index continued to demonstrate the greatest diagnostic utility, with an improved Youden index value of 71.6%. This was followed by RDWI, Mentzer, England & Fraser, Sirvastava, and Shin & Lal indices, in that order.

## Discussion

IDA and BTT are the most prevalent causes of microcytic hypochromic anemia [15]. Beta-thalassemia is a hereditary disorder that impairs the synthesis of the beta-globin chains in hemoglobin [18–21]. BTT, a common cause of microcytic anemia, typically does not require treatment. However, early screening and accurate diagnosis are essential, particularly in premarital settings, to prevent the birth of children with thalassemia major in couples who are both carriers of the trait [22].

Given the high prevalence of both IDA and BTT in Sistan and Baluchestan Province, along with the high rate of consanguineous marriages, the accurate diagnosis of BTT is of paramount importance for public health [2]. A definitive distinction between IDA and BTT can

**Table 1.** Hematological indices of IDA (n=74) and BTT (n=74) groups

Index	Groups	Mean $\pm$ SD	P
WBC ( $\times 10^9$ /L)	IDA	9.33 $\pm$ 3.09	0.149
	BTT	8.63 $\pm$ 2.73	
RBC ( $\times 10^{12}$ /L)	IDA	4.64 $\pm$ 0.42	0.000
	BTT	5.55 $\pm$ 0.56	
HB (g/dL)	IDA	9.69 $\pm$ 1.03	0.001
	BTT	10.21 $\pm$ 0.75	
HCT (%)	IDA	31.38 $\pm$ 4.2	0.000
	BTT	34 $\pm$ 3.14	
MCV (fL)	IDA	67.73 $\pm$ 6.63	0.000
	BTT	62.1 $\pm$ 4.63	
MCH (pg)	IDA	21.02 $\pm$ 2.9	0.000
	BTT	18.94 $\pm$ 2.08	
MCHC (g/dL)	IDA	30.06 $\pm$ 2.27	0.487
	BTT	30.29 $\pm$ 1.82	
PLT ( $\times 10^3$ /mL)	IDA	379.31 $\pm$ 143.19	0.453
	BTT	363.6 $\pm$ 108.62	
MPV (fL)	IDA	8.37 $\pm$ 0.94	0.064
	BTT	8.66 $\pm$ 0.91	
RDW (%)	IDA	16.77 $\pm$ 2.71	0.458
	BTT	16.43 $\pm$ 2.81	

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Abbreviations: IDA: Iron deficiency anemia; BTT: Beta thalassemia trait; WBC: White blood cell; RBC: Red blood cell; Hb: Hemoglobin; HCT: Hematocrit; MCV: Mean cell volume; MCH: Mean cell hemoglobin; MCHC: Mean cell hemoglobin concentration; PLT: Platelet; MPV: Mean platelet volume; RDW: Red distribution width.

be made using a range of diagnostic markers, including HbA2, serum iron, ferritin, and total iron-binding capacity (TIBC) levels [23].

Although several differential diagnostic indices have been proposed to distinguish between BTT and IDA, none has demonstrated perfect sensitivity or specificity [11–13, 24]. In our study population, the RBC count was found to be the most reliable and accurate index for differential diagnosis, yielding the highest Youden index. After optimizing the threshold, the RBC count index reached a Youden index of 71.6%, followed by RDWI, Mentzer, England & Fraser, Sirvastava, and Shin & Lal indices.

In a study conducted by Keikhaei et al., involving 173 patients with IDA and 150 with BTT, Shin & Lal and RBC count were identified as the most accurate indices for children under 10 years old, whereas RDWI and RBC count were most effective for those over 10 years [12]. Similarly, in a study by Ehsani et al. involving 284 patients (130 with IDA and 154 with BTT), the Mentzer index, their newly proposed index, and RBC count all demonstrated diagnostic accuracies exceeding 90% [11]. Another study of 63 children (26 with IDA and 37 with BTT) found RDWI and RBC count to be the most reliable indices, with diagnostic accuracies above 90% for RBC count, RDWI, and Mentzer index [24].

**Table 2.** The value of indices in differentiating IDA and BTT patients

Differentiate Index	Value	No.		Patients With Correct Diagnosis	
		BTT (n=74)	IDA (n=74)	No.	%
Mentzer (MCV/RBC)	IDA>13	13	60	61+60=121	81.75
	BTT<13	61	14		
Shin & Lal (MCV <sup>2</sup> × MCH × 0.01)	IDA>1530	0	1	74+1=75	50.67
	BTT<1530	74	73		
Sirvastava (MCH/RBC)	IDA>3.8	18	62	56+62=118	79.72
	BTT<3.8	56	12		
England & Fraser (MCV-RBC-5HB-3.4)	IDA>0	51	73	23+73=96	64.86
	BTT<0	23	1		
RDWI (MCV × RDW/RBC)	IDA>220	11	60	63+60=123	83.1
	BTT<220	63	14		
RBC count	IDA<5	10	60	64+60=124	83.78
	BTT>5	64	14		

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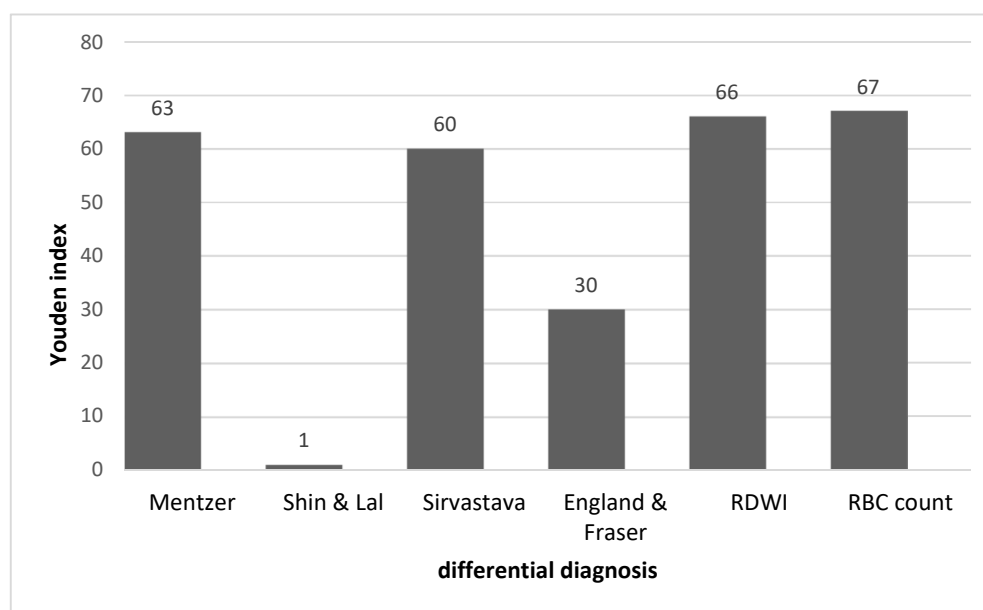
Abbreviations: IDA: Iron deficiency anemia; BTT: Beta thalassemia trait; WBC: White blood cell; RBC: Red blood cell; Hb: Hemoglobin; MCV: Mean cell volume; MCH: Mean cell hemoglobin; RDW: Red distribution width.

**Table 3.** ROC curve analysis

Differentiate Index	Threshold	Sensitivity	Specificity	Youden Index	AUC	Best Threshold
Mentzer	12.69	83.8	79.7	63.5	0.880	12.75
	12.75	83.8	81.1	64.9		
	13.09	81.01	83.8	64.9		
Shin & Lal	754	78.4	67.6	46	0.779	782
	782	75.7	71.6	47.3		
	785	74.3	71.6	45.9		
Sirvastava	3.58	87.8	73	60.8	0.841	3.9
	3.9	83.8	77	60.8		
	4.03	78.4	82.4	60.8		
England & Fraser	5.6	83.8	78.4	62.2	0.881	6.2
	6.2	81.1	81.1	62.2		
	4.6	90.5	70.3	60.8		
Red cell distribution width	212	86.5	83.8	70.3	0.887	214
	214	86.5	85.1	71.6		
	216	85.1	85.1	70.2		
RBC count	5.01	86.5	83.8	70.3	0.109	5.05
	5.05	83.8	87.8	71.6		
	5.07	82.4	89.2	71.6		

AUC: Area under the curve.

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**Figure 1.** Youden index of diagnostic indices in the differential diagnosis of IDA and BTT

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Abbreviations: IDA: Iron deficiency anemia; BTT: Beta thalassemia trait; RBC: Red blood cell; RDW: Red distribution width.

Sirdah et al. studied 2,196 Palestinian patients with microcytic anemia, including 1,272 with BTT and 924 with IDA. The diagnostic thresholds identified in their study were: Mentzer index=12.88, Shin & Lal=1130, England & Fraser=-1.79, RDWI=214, and Sirvastava=4.1 [13]. In our study, the corresponding thresholds were: Mentzer index=12.75, Shin & Lal=782, England & Fraser=6.2, RDWI=214, and Sirvastava=3.9. Notably, the threshold for RDWI was consistent with that of Sirdah's study.

Despite the widespread use of these indices, none has demonstrated perfect sensitivity and specificity, and findings vary considerably across studies. For instance, Keikhaei et al. identified RBC count as the most reliable and accurate index based on the Youden index [12], while Okan et al. found Shin & Lal to be the best [14]. Ehsani et al. emphasized the Mentzer index [11], and Demir et al. highlighted RBC count and RDWI as the most accurate [24]. Ahmadi et al. also concluded that Shin & Lal was the most effective index according to the Youden index [25].

In our study, none of the evaluated indices demonstrated perfect diagnostic performance. However, Shin & Lal achieved 100% sensitivity for BTT detection, and England & Fraser showed 99% sensitivity for IDA—though both exhibited relatively low specificity. The three indices with the highest overall diagnostic performance, based on the Youden index, were RBC count, RDWI, and Mentzer, respectively. After recalculating thresholds for

improved accuracy, the England & Fraser index achieved both sensitivity and specificity above 80%, ranking just below the Mentzer index.

Variability in results across different studies may be attributed to differences in patient inclusion and exclusion criteria. Factors such as age distribution, sex, HbA2 and ferritin levels, hemoglobin concentration, MCV, and IDA severity can significantly affect diagnostic accuracy. In this study, we attempted to minimize these discrepancies by limiting the age range and calibrating RBC index thresholds according to age and sex. Nonetheless, we recommend that future studies stratify participants by age, sex, and severity of anemia. Furthermore, participants should be screened for recent drug or iron supplement use. Efforts should also be made to standardize the performance and calibration of hematology analyzers, as variations in equipment and laboratory protocols can influence diagnostic accuracy.

## Conclusion

Based on the findings of this study, the RBC count is a relatively reliable, simple, and accessible index for differentiating between IDA and BTT. Nevertheless, it is important to consider additional diagnostic indices and clinical parameters to ensure accurate and comprehensive evaluation.



## Ethical Considerations

### Compliance with ethical guidelines

The study protocol was approved by the Ethics Committee of [Zahedan University of Medical Sciences](#), Zahedan, Iran (Code: IR.ZAUMS.REC.1393.1157), and informed consent was obtained from all participants or their legal guardians.

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### Authors contributions

Conceptualisation and study design: Ghasem Miri-Aliabad; Data acquisition and statistical analysis: Saeed Sedaghatyan; Writing: Ghasem Miri-Aliabad and Leila Asgarzadeh; Supervision: Ghasem Miri-Aliabad.

### Conflicts of interest

The authors declared no conflict of interests.

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