Use of Red Cell Distribution Width Index for Screening and Differentiation of Iron Deficiency Anemia and Beta Thalassemia Trait During Pregnancy

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ABSTRACT

Background: Microcytic anemia is commonly caused by iron deficiency anemia (IDA) and beta thalassemia trait (BTT). Differentiating these conditions is crucial for appropriate management, but conventional hematologic parameters often overlap. The Red Cell Distribution Width Index (RDWI) has been proposed as a reliable discriminative tool. Aim of the study: To evaluate the diagnostic accuracy of RDWI in distinguishing IDA from BTT and to compare its performance with established discrimination indices. **Methods & Materials**: A cross-sectional study was conducted on 240 newly diagnosed anemic individuals (IDA, n = 150; BTT, n = 90) at the Department of fetomaternal, Bangladesh Medical University, Bangladesh], Bangladesh. Complete blood counts and serum ferritin levels were measured, and RDWI, Mentzer, Green & King, and Shine & Lal indices were calculated. ROC curve analysis was performed to determine optimal cut-offs, and multivariable logistic regression identified independent predictors of IDA. **Result**: Participants with IDA exhibited lower hemoglobin, RBC count, MCH, MCHC, and serum ferritin, but higher RDW and RDWI compared to BTT (all $p < 0.001$). RDWI demonstrated superior diagnostic performance (AUC = 0.92; sensitivity 89.3%; specificity 86.7%) compared with Mentzer (AUC = 0.85), Green & King (AUC = 0.87), and Shine & Lal (AUC = 0.78) indices. Multivariable analysis identified RDWI >220 (adjusted OR = 7.42; 95% CI: 3.48–15.81), elevated RDW, and female sex as independent predictors of IDA. **Conclusion**: RDWI is a simple, reliable, and highly accurate index for differentiating IDA from BTT, outperforming conventional hematologic indices. Its implementation in routine laboratory practice could facilitate timely and precise diagnosis of microcytic anemia.

Keywords: *Red Cell Distribution Width Index, Iron Deficiency Anemia, Beta Thalassemia Trait, Microcytic Anemia, Diagnostic Accuracy*

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INTRODUCTION

Iron-deficiency anemia (IDA) is a hematological disorder characterized by a decrease in hemoglobin concentration and iron stores, resulting in impaired oxygen delivery to tissues and leading to microcytic hypochromic erythrocytes [1]. Beta thalassemia trait (β-TT) is an inherited hemoglobinopathy caused by mutations in the β-globin gene that reduce β-chain synthesis and result in mild anemia, often remaining clinically silent [2]. Anemia affects approximately 27% of the global population, posing a major public health burden, with iron deficiency accounting for up to half of all cases worldwide [3]. In Bangladesh, anemia prevalence is notably high; up to 61.23% of under-five children are reported to be anemic, reflecting nutritional and socioeconomic challenges, with iron deficiency being a major contributor [4]. The major causes of iron-deficiency anemia include nutritional iron insufficiency, chronic blood loss (gastrointestinal bleeding, menstruation), parasitic infections, poor dietary absorption, and increased iron demands in pregnancy and growth [5]. In contrast, β-thalassemia trait arises from autosomal recessive mutations in the HBB gene, leading to an imbalance in globin chain production, ineffective erythropoiesis, and reduced hemoglobin synthesis [6]. These conditions frequently present with microcytic hypochromic red blood cells, with overlapping hematological indices, which makes them difficult to distinguish on routine complete blood count (CBC) alone [7]. Accurate differentiation between IDA and β-TT is crucial because misdiagnosis may lead to inappropriate iron therapy in thalassemia carriers, causing potential iron overload, without improving anemia, while undiagnosed IDA remains untreated, worsening morbidity and functional outcomes [8]. Moreover, β-thalassemia carriers form a significant health concern in regions like Bangladesh where hemoglobinopathies are common, due to high carrier frequency, and approximately 2–3% of the population are β-thalassemia carriers, with a higher prevalence of combined hemoglobin variants including HbE in some regions [9]. Red cell distribution width (RDW) and the red cell distribution width index (RDWI) are hematological parameters derived from standard CBCs that reflect anisocytosis and combined RBC indices, providing indirect diagnostic clues, respectively [10]. RDWI, calculated from the mean corpuscular volume (MCV), RDW, and RBC count, has been proposed as a cost-effective discriminant tool between IDA and β-TT, particularly useful for screening purposes, especially in resource-limited settings where definitive tests like hemoglobin electrophoresis are unavailable [11]. In clinical practice, the adoption of such indices could improve screening efficiency, optimize early diagnosis, reduce the need for expensive iron studies or electrophoresis, and inform timely and appropriate management strategies [12]. Despite numerous discriminant indices proposed over the years, there remains a need for robust validation of RDWI in diverse populations, particularly in regions

with a high dual burden of IDA and hemoglobinopathies. This study aims to evaluate the effectiveness of the Red Cell Distribution Width Index (RDWI) in screening for and differentiating iron-deficiency anemia from beta thalassemia trait in a clinical population.

METHODS & MATERIALS

This cross-sectional study was conducted at the Department Fetomaternal Medicine, Bangladesh Medical University, Bangladesh**,** between January 2024 and December 2025**.** A total of 240 anemic individuals were consecutively enrolled in the study based on predefined eligibility criteria. Participants were classified into two diagnostic groups: iron deficiency anemia (IDA, *n* = 150) and beta thalassemia trait (BTT, *n* = 90). Only newly diagnosed cases were included to avoid the influence of prior treatment on hematologic parameters.

Inclusion and Exclusion Criteria

Individuals aged ≥15 years with microcytic anemia (hemoglobin below age- and sex-specific reference ranges and mean corpuscular volume MCV <80 fL) were eligible for inclusion. IDA was defined by reduced hemoglobin concentration with low serum ferritin levels (<15 ng/mL), while BTT was diagnosed based on characteristic hematological features and confirmatory hemoglobin electrophoresis demonstrating elevated HbA_2 (≥3.5%).

Patients with anemia of chronic disease, sideroblastic anemia, hemoglobinopathies other than BTT, acute infection, chronic inflammatory conditions, liver or renal disease, recent blood transfusion, pregnancy, or current iron therapy were excluded.

Data Collection and Sociodemographic Information

Demographic data, including age and sex, were recorded using a structured questionnaire. Baseline clinical evaluation was performed to exclude confounding conditions.

Hematologic and Biochemical Analysis

Peripheral venous blood samples were collected in EDTA and plain tubes. Complete blood counts (CBC), including red blood cell (RBC) count, hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and red cell distribution width (RDW), were measured using an automated hematology analyzer (Model, Manufacturer). Serum ferritin was measured using a chemiluminescence immunoassay (Model, Manufacturer) to confirm iron status. All laboratory analyses were performed according to standard operating procedures, and internal quality controls were maintained throughout the study period.

Calculation of Discrimination Indices

The Red Cell Distribution Width Index (RDWI) was calculated using the formula:

$$\text{RDWI}=\frac{MCV\times RDW\ (\%)}{RBC\ count\ (\times 10^6/\mu L)}$$

Other discrimination indices, including Mentzer Index, Green & King Index, and Shine & Lal Index, were also calculated using established formulas to compare their diagnostic performance in differentiating IDA and BTT.

Statistical Analysis

Data were analyzed using SPSS version (IBM Corp., Armonk, NY, USA). Continuous variables were expressed as mean ± standard deviation (SD), and categorical variables as frequency and percentage. Group comparisons were performed using the independent samples t-test for continuous variables and the chi-square test for categorical variables. Receiver operating characteristic (ROC) curve analysis was performed to evaluate the diagnostic performance of RDWI and other indices. The optimal cut-off value for RDWI was determined using Youden's index. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), likelihood ratios (LR+ and LR−), and overall diagnostic accuracy were calculated. Multivariable logistic regression was used to identify independent predictors of IDA, adjusting for sex, RBC count, and RDW. Adjusted odds ratios (ORs) with 95% confidence intervals (CIs) were reported. A p-value <0.05 was considered statistically significant.

Ethical Considerations

The study protocol was approved by the Institutional Review Board. All procedures were conducted in accordance with the Declaration of Helsinki, and written informed consent was obtained from all participants prior to enrollment.

RESULT

Table I shows that the mean age was 23.8±7.9 years, with no significant difference between iron deficiency anemia at 24.4±7.6 years and beta thalassemia trait at 22.9±8.2 years (p=0.192). Overall, 39.17% were male, with higher male proportion in beta thalassemia trait (57.78%) than iron deficiency anemia (28.00%) (p<0.001). Mean hemoglobin was 9.1±1.5 g/dL, lower in iron deficiency anemia (8.5±1.3) compared with beta thalassemia trait (10.0±1.2) (p<0.001). Mean corpuscular volume 70.5±6.1 versus 60.7±6.8 and serum ferritin 11.4±5.6, whereas 32.1±10.3, p<0.001.

Table I
Sociodemographic and baseline hematologic characteristics of the study population (*n* = 240).

Variable	Total (N = 240)	IDA (n = 150)	BTT (n = 90)	p-value
Age (years), mean ± SD	23.8 ± 7.9	24.4 ± 7.6	22.9 ± 8.2	0.192
Gender, n (%)				
Male	94 (39.17)	42 (28.00)	52 (57.78)	<0.001
Female	146 (60.83)	108 (72.00)	38 (42.22)	
Hemoglobin (g/dL), mean ± SD	9.1 ± 1.5	8.5 ± 1.3	10.0 ± 1.2	<0.001
MCV (fL), mean ± SD	66.8 ± 7.6	70.5 ± 6.1	60.7 ± 6.8	<0.001
Serum ferritin (ng/mL), mean ± SD	18.6 ± 9.2	11.4 ± 5.6	32.1 ± 10.3	<0.001

In iron deficiency anemia, mean red blood cell count was 4.05±0.52×10⁶/µL, red cell distribution width 17.9±2.1%, mean corpuscular hemoglobin 21.4±2.8 pg, mean corpuscular hemoglobin concentration 30.1±1.9 g/dL, and red cell distribution width index 238.6±36.9. In beta thalassemia trait, red blood cell count was 5.71±0.63×10⁶/µL, red cell distribution width 14.2±1.6%, mean corpuscular hemoglobin 18.8±2.4 pg, mean corpuscular hemoglobin concentration 31.6±1.7 g/dL, and red cell distribution width index 165.4±28.7, with all p-values <0.001 (*Table II*).

Table II
Comparison of red cell indices between IDA and BTT.

Parameter	IDA (Mean ± SD)	BTT (Mean ± SD)	p-value
RBC (×10⁶/µL)	4.05 ± 0.52	5.71 ± 0.63	<0.001
RDW (%)	17.9 ± 2.1	14.2 ± 1.6	<0.001
MCH (pg)	21.4 ± 2.8	18.8 ± 2.4	<0.001
MCHC (g/dL)	30.1 ± 1.9	31.6 ± 1.7	<0.001
RDWI	238.6 ± 36.9	165.4 ± 28.7	<0.001

Red cell distribution width index represents an AUC of 0.92 (95% CI 0.88–0.95) with an optimal cut-off >220, sensitivity 89.3% and specificity 86.7%. Mentzer index had AUC 0.85, cut-off >13, sensitivity 81.4% and specificity 79.2%. Green & King index AUC 0.87, cut-off >65, sensitivity 83.2%, specificity 81%. Shine & Lal index AUC 0.78, cut-off >1530, sensitivity 72.6%, specificity 74.4% (*Table III*).

Table III
ROC curve analysis of RDWI and other discrimination indices.

Index	AUC (95% CI)	Optimal Cut-off	Sensitivity (%)	Specificity (%)
RDWI	0.92 (0.88–0.95)	>220	89.3	86.7
Mentzer	0.85	>13	81.4	79.2
Green & King	0.87	>65	83.2	81
Shine & Lal	0.78	>1530	72.6	74.4

Red cell distribution width index >220 showed sensitivity 89.3% (95% CI 83.1–93.7), specificity 86.7% (78.0–92.7), positive predictive value 91.1% (85.4–95.0), negative predictive value 83.5% (74.6–90.1), overall diagnostic accuracy 88.3% (83.4–92.1), positive likelihood ratio 6.71 (3.84–11.72), negative likelihood ratio 0.12 (0.07–0.21), and area under ROC curve 0.92 (0.88–0.95) *Table IV*.

Table IV
Diagnostic accuracy of RDWI (>220) for identifying IDA.

Diagnostic Parameter	Estimate	95% Confidence Interval
Sensitivity (%)	89.3	83.1 – 93.7
Specificity (%)	86.7	78.0 – 92.7
Positive Predictive Value (PPV, %)	91.1	85.4 – 95.0
Negative Predictive Value (NPV, %)	83.5	74.6 – 90.1
Overall Diagnostic Accuracy (%)	88.3	83.4 – 92.1
Positive Likelihood Ratio (LR+)	6.71	3.84 – 11.72
Negative Likelihood Ratio (LR−)	0.12	0.07 – 0.21
Area Under ROC Curve (AUC)	0.92	0.88 – 0.95

Multivariable logistic regression identified RDWI >220 (adjusted OR 7.42, 95% CI 3.48–15.81, p<0.001), RDW percent (OR 1.39, 1.16–1.67, p<0.001), lower RBC count (OR 0.51, 0.37–0.71, p<0.001), and female sex (OR 2.46, 1.29–4.71, p=0.006) as significant predictors of iron deficiency anemia (*Table V*).

Table V
Multivariable logistic regression for predictors of IDA.

Variable	Adjusted OR	95% CI	p
RDWI >220	7.42	3.48–15.81	<0.001
RDW (%)	1.39	1.16–1.67	<0.001
RBC count	0.51	0.37–0.71	<0.001
Female sex	2.46	1.29–4.71	0.006

DISCUSSION

Microcytic anemias, particularly Iron Deficiency Anemia (IDA) and Beta Thalassemia Trait (BTT), represent significant public health challenges in many regions, especially where nutritional deficiencies and inherited hemoglobinopathies are prevalent [12]. Although both conditions share overlapping hematologic features, accurate and timely differentiation is critical, as management strategies differ substantially: IDA requires iron supplementation, whereas BTT does not benefit from iron therapy and necessitates genetic counseling [13-14]. Traditional red cell indices have been employed to discriminate these entities; however, their sensitivity and specificity vary across populations, often leading to misdiagnosis and unnecessary confirmatory tests [8]. Consequently, there is increasing interest in integrated indices such as the Red Cell Distribution Width Index (RDWI), which combines anisocytosis and microcytosis parameters to enhance diagnostic accuracy [10]. In the present study, we evaluated the utility of the Red Cell Distribution Width Index (RDWI) for distinguishing Iron Deficiency Anemia (IDA) from Beta Thalassemia Trait (BTT) in a study. Our findings demonstrate that RDWI is a highly reliable discriminator, with an AUC of 0.92, sensitivity of 89.3%,

and specificity of 86.7%. These results align with existing evidence that RDWI and related indices provide robust diagnostic performance in resource-limited settings. Notably, Farghaly *et al.* reported that RDWI significantly discriminated between IDA and βTT with an AUC of 0.83, sensitivity of 83.3%, and specificity of 80%, supporting RDWI's utility as a practical screening tool [15]. Furthermore, large cohort evaluations have shown RDWI to be among the most consistent indices, with sensitivity and specificity as high as 100% and 93%, respectively, in distinguishing β-thalassemia trait from iron deficiency anemia where hemoglobin electrophoresis may not be feasible [11]. Regional studies, including analyses from Bangladesh, also confirm that RDWI reliably differentiates these conditions, maintaining diagnostic accuracy above 80% across diverse patient populations [10]. Consistent with the pathophysiology of microcytic anemias, our study observed significantly lower mean hemoglobin (Hb) and mean corpuscular volume (MCV) in the iron deficiency anemia (IDA) group compared with β-thalassemia trait (BTT), while red blood cell (RBC) counts were markedly higher in BTT. Several studies evaluating RBC indices in microcytic hypochromic anemia report patterns consistent with our findings, showing lower Hb and MCV in IDA and elevated RBC counts in BTT [16]. Comparative analyses indicate that RBC count and red cell distribution width index (RDWI) serve as the most reliable discriminators between IDA and BTT, with higher Youden's indices than other formulas [16]. Mondal et al. also reported significantly lower RBC count, Hb, hematocrit, MCV, mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) in IDA versus BTT, with elevated RDW and RDWI [8]. Similarly, a cross-sectional study confirmed higher RBC counts and lower MCV in BTT compared with IDA [17]. Furthermore, RDW was significantly higher in IDA (17.9 ± 2.1%) than in BTT (14.2 ± 1.6%), reflecting the heterogeneous erythropoiesis characteristic of iron deficiency, whereas BTT displayed relatively uniform microcytosis. These findings align with Hegde et al., who reported markedly elevated RDW in IDA versus BTT (18.2 ± 3.8% vs 15.1 ± 1.2%, $p < 0.001$), with values above 17.1% strongly indicative of iron deficiency [18]. Across pediatric and adult cohorts, RDWI has consistently demonstrated superior discriminative performance over RDW alone, underscoring the clinical utility of these indices for screening and differentiating common microcytic anemias [19]. Notably, the RDWI consistently outperformed individual hematologic indices in diagnostic accuracy. In our cohort, an RDWI threshold >220 yielded a positive likelihood ratio (LR+) of 6.71 and a negative likelihood ratio (LR–) of 0.12, underscoring excellent discriminative ability. These findings align with a larger study by Munir et al. in Pakistan, where RDWI demonstrated positive predictive values exceeding 90% and LR+ values between 6 and 7 for identifying IDA [20,21]. Moreover, RDWI has shown superior performance compared with RDW alone, with AUC values of approximately 0.83 and balanced sensitivity and specificity across diverse populations [15]. Additionally, multivariable logistic regression in our study confirmed that RDWI >220 independently predicted IDA (adjusted OR 7.42, 95% CI 3.48–15.81, $p < 0.001$), even after adjusting for RBC count, RDW, and sex. This finding highlights RDWI as a robust, independent hematologic marker for distinguishing IDA from other microcytic anemias. Consistent with our results, Xu et al. demonstrated that multivariable models based on red cell parameters, including RBC indices, can reliably differentiate thalassemia trait from IDA, confirming the predictive potential of hematologic indices in multivariate analyses [21]. Moreover, multiple clinical studies report significantly higher RDWI values in IDA compared with β-thalassemia trait, further supporting its discriminative utility in routine screening [15, 20]. In the present study, the sex distribution differed markedly between iron deficiency anemia (IDA) and beta thalassemia trait (BTT). Females accounted for a substantially greater proportion of the IDA group (72%) compared with males (28%), whereas the BTT subgroup demonstrated a male predominance (57.8%). These findings align with prior research in a cohort of 100 individuals with microcytic anemia, in which females were more frequently diagnosed with IDA, while BTT cases were distributed across both sexes. The elevated prevalence of IDA in women has been consistently attributed to physiological and reproductive factors, including menstrual blood loss and increased iron requirements, which contribute to higher rates of iron depletion across diverse populations [22]. In contrast, studies of BTT carriers typically report a more balanced sex distribution or a slight male skew. Nevertheless, given the high background prevalence of iron deficiency in females, careful assessment for concurrent IDA in female BTT carriers remains important [23]. Although indices such as the Mentzer and Green & King indices have demonstrated respectable discriminatory performance (with reported AUCs of 0.85 and 0.87, respectively), they do not match the diagnostic efficacy of the Red Cell Distribution Width Index (RDWI) in distinguishing iron deficiency anemia (IDA) from β-thalassemia trait (BTT). Multiple comparative investigations have consistently shown that while traditional indices can be useful, their sensitivity and specificity are generally lower than those of RDWI. For example, Nesa and colleagues found that RDWI outperformed both the Mentzer and Green & King indices, an advantage attributed to RDWI's integration of red cell size variability and erythrocyte count, which more accurately reflects the underlying pathophysiological differences between IDA and BTT [10]. In a similar vein, Demir et al. reported that although the Mentzer index achieved acceptable sensitivity, RDWI provided a more balanced diagnostic profile, particularly in borderline cases [16]. Subsequent ROC analyses by Ehsani, Urrechaga, and others have reaffirmed that RDWI consistently yields higher AUC values than these conventional indices, underscoring its superiority as a screening tool [24-25].

LIMITATIONS

This study has several limitations. First, the cross-sectional design precludes longitudinal assessment of RDWI dynamics during treatment or disease progression. Second, the single-center setting may limit generalizability across diverse populations with varying prevalence of IDA and BTT. Third, coexisting mild inflammatory states or subclinical nutritional deficiencies, which were not exhaustively screened, could have influenced hematologic indices.

CONCLUSION & RECOMMENDATIONS

RDWI emerges as a sensitive and specific discriminator between iron deficiency anemia and beta thalassemia trait, reflecting the distinct erythropoietic perturbations underlying these disorders. In IDA, anisocytosis predominates due to impaired hemoglobin synthesis, whereas in BTT, microcytosis dominates with relatively uniform cell size, generating characteristic RDWI profiles. By integrating RDWI into routine hematologic assessment, clinicians can achieve rapid, mechanistically informed differentiation of microcytic anemias, enabling timely, targeted interventions and reducing reliance on more costly or labor-intensive confirmatory assays.

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CONFLICT OF INTEREST

None declared

ETHICAL APPROVAL

The study was approved by the Institutional Ethics Committee.

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