Red Cell Distribution Width Index versus Red Cell Distribution Width as a Discriminating Guide for Iron Deficiency Anemia and Beta Thalassemia Trait

Hekma Saad Farghaly, Mervat Amin Mahmoud, Rania Eshak Gedy*
Pediatric Department, Assiut University Hospital, Assiut, Egypt.
Pediatric Department, Assiut Police Hospital*, Assiut, Egypt.
***Corresponding Author*:** Rania Eshak Gedy
E-mail: *Raniaeshak2016@gmail.com*

Abstract:

Introduction: Iron deficiency anemia (IDA) and β-thalassemia trait (βTT) are considered to be the two main causes of microcytic hypochromic anaemias. RDWI can be easily calculated as (MCVXRDW/RBC) and can be a reliable index in differentiation between βTT and IDA.

Aim of Work: Our study aimed to evaluate RDW and RDWI in the differentiation between iron deficiency anemia and beta thalassemia trait.

Patient and Methods: This study was conducted in the hematology outpatient clinic, Assuit University, Children's Hospital. One hundred and twenty children with microcytic hypochromic anemia were enrolled in this study. Sixty children diagnosed with Iron deficiency anemia (IDA), 60 children previously diagnosed with beta thalassemia trait (βTT) by hemoglobin electrophoresis, and followed up in the hematology unit.

Results: RDWI could significantly discriminate between IDA and βTT with an area under the curve equal to 0.83 (P-value < 0.001) with 83.3% sensitivity and 80% specificity. There were significant differences between the IDA and βTT groups in RDW and RDWI, with the P-value <.05being higher in IDA. Significant differences existed between the two groups in S. iron, TIBC, and S. ferritin (p-value =0.000).

Conclusion: Red cell distribution index (RDWI) is more reliable than RDW for differentiation between IDA and βTT, and it is easy, cheap, and a more accessible parameter without being time-consuming.

Keywords: Microcytic anemias; IDA; βTT; RDWI.

Introduction:

Microcytic hypochromic anaemia is a very common hematological abnormality in clinical practice. Iron deficiency anaemia and beta thalassemia trait are the most common causes of microcytic hypochromic anaemias **(1)**.

The differentiation between them is crucial for two reasons. First, hemoglobin will not improve in beta thalassemia traits if it is misdiagnosed as iron deficiency anemia, and unnecessary iron is prescribed ***(2).*** The second reason is that misdiagnosing beta thalassemia as Iron deficiency anemia may result in a beta thalassemia trait, resulting in homozygous or thalassemia major in the offspring ***(3).*** One needs a lot of tests, including peripheral blood picture, HBA2 estimation, serum iron, TIBC, serum ferritin, and transferrin saturation to differentiate Iron deficiency anemia from beta thalassemia trait clearly ***(4).*** RDW is the first index to become abnormal during the development of Iron deficiency anemia***(5).*** It quantitatively estimates red blood cell size variation computed directly from the RBCs histogram and is calculated as a standard statistical value, the coefficient of variation of the volume distribution ***(6).***

RDW is high in IDA due to the wide variation in red cell size. In BTT, the red

cells are all the same size, so RDW is low ***(7).*** Another red cell discriminant function, RDWI, which is calculated as (MCV in (Fl) x RDW / RBCs in (million per microlitre)), provides valuable help as all discriminating factors, including RBCs count, MCV, and RDW, are involved in its formula ***(8).***

Our study aimed to evaluate RDW and RDWI in differentiating between iron deficiency anemia and beta thalassemia trait

Patients and Methods

Study Populations

This cross-sectional study was conducted in the hematology outpatient clinic, Children's Hospital, Assuit University. One hundred and twenty children with microcytic hypochromic anemia were enrolled in this study. Sixty children diagnosed with Iron deficiency anemia (IDA) were recruited before starting iron therapy, and 60 children previously diagnosed with beta thalassemia trait (βTT) by hemoglobin electrophoresis were followed up in the haematology outpatients clinic.

Inclusion Criteria

All patients with microcytic hypochromic anemia (According to WHO, MCV is less than 80 fL, and Hb level is below the limit of normal value specified by age and gender ***(9).***

Groups by age and gender	HB level (gm/dl)
Children aged between 5 and 11 years old	11.5
Children aged between 12 and 14 years old	12
Girls aged more than 15 years old	12
Boys aged more than 15 years old	13

Exclusion criteria:

Beta thalassemia major patients.
Chronic diseases or infections.
Lead poisoning.
Sideroblastic anemia.

Demographic data

History was taken from Children who fulfill the criteria, including age, gender, and residency.

Laboratory data

a) Venous blood samples were obtained after children had a 30-minute rest sitting. Two blood samples were taken from each child, one sample used for a complete blood picture including the following parameters (Hemoglobin(Hb), RBCs count, reticulocytic count (retics), Mean corpuscular volume(MCV), mean corpuscular hemoglobin concentration (MCHC), and Red cell distribution width (RDW). The other sample was collected and centrifuged for 5 minutes to get the serum. The serum was used for serum iron, ferritin, and total iron binding capacity (TIBC).

b) Patients diagnosed as BTT if the hemoglobin electrophoresis showed HbA2 more than 3.2% were anaemic patients with serum ferritin less than 12ng/ml were identified as IDA cases ***(10).***

c) Red cell distribution width index (RDWI) was calculated using this formula: RDWI=MCV XRDW/RBC ***(11).***

Interpretation: IDA:>220 , BTT:<220

d) Red cell distribution width (RDW) was calculated as a standard statistical value, the coefficient of variation of the volume distribution ***(12).***

Interpretation: IDA:>14, BTT:<14.

Ethical Considerations

The work was approved by the Ethical Committee of the Faculty of Medicine of Assiut University (approval number 17100700). Informed consent was obtained from the parents of the patients before their recruitment into the study. Refusal would not affect medical services, which are usually offered.

Statistical Analysis

Data analysis was done using the Statistical Package for the Social Sciences (SPSS), version 20. Data were presented as

means ± standard deviation (SD) or median (IQR) for quantitative data and as numbers with percentages for qualitative data.

Normality of quantitative data distribution was examined using the Kolmogorov-Smirnov test. The t-test or Mann-Whitney U test determined statistical associations between quantitative data. The chi-square test was used for qualitative data. Validity of the discrimination indices in differentiation of BTT and IDA was evaluated by calculating their sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and Youden's index (YI). A P value <0.05 was considered statistically significant, < 0.01highly significant, and < 0.001 highly significant.

Results

The distribution of the gender was 86 (71.7%) males and 34 (28.3%) females, and the mean age was 6±3.5 years (ranging from 1 year to 16 years old). This study showed that the gender distribution and the age are not significantly different between the two studied groups (p-value > 0.05). **(Table 1)**

CBC analysis **(Table 2)** showed that HgB, MCV, MCHC, and MCH were significantly higher in the IDA group compared to the BTT group. Also, RDW **(Figure 1)** and RWDI **(Figure 2)** were significantly higher in the IDA group. However, RBCs and reticulocytic counts were significantly higher in the BTT group. No significant difference regarding WBCs counts could be detected between groups **(Table 2)**.

Iron study for both groups is shown in **Table 3**. Serum iron and Serum ferritin were significantly lower in IDA patients, while TIBC was significantly higher compared to the BTT group.

The Validity of the discrimination indices between BTT and IDA is shown in **Table 4** . RDWI was more sensitive and specific (83.33%, 80% respectively for IDA and 80%, 83.33% respectively for BTT) than RDW (80%, 66.67% respectively for IDA, and 66.67%, 80% respectively for BTT with higher Youden's index 63.33 at cut-off value <220 for BTT. ROC curve illustrated that RDWI could significantly discriminate between IDA and BTT with an area under the curve equal to 0.83, compared to IDA(area under the curve equal to 0.732) with a P-value< 0.001.

RDWI is better discriminant than RDW, whereas Youden's index is higher in RDWI.

Discussion

Disorders interfering with the formation and rate of production of hemoglobin (Hb) can induce a reduction in mean red cell Hgb and corpuscular volume (MCV) with resultant hypochromia and microcytosis ***(13).***

The commonest single nutrient disease in humans is IDA, which causes microcytic hypochromic anemia ***(14).*** Microcytic anemia in the case of thalassemia results from impaired globin chain synthesis and decreased hemoglobin (HbA1) synthesis***(15).***

The differentiation between βTT and IDA requires HbA2 analysis by Hb electrophoresis, peripheral blood film, serum ferritin, iron, TIBC, and transferrin saturation. However, being relatively expensive and time-consuming, it is necessary to find another simple parameter. Red cell distribution width (RDW) is automatically provided in CBC and can be utilized with a derived value, RDWI, to discriminate IDA and βTT ***(6).*** Red cell distribution width index (RDWI) is a reliable discrimination index in differentiation between IDA and BTT, which one can be calculated as (MCVXRDW/RBC) ***(16).***

In the present study, we evaluated the accuracy of RDW and RDWI in the differentiation between iron deficiency anemia and beta thalassemia traits.

There was a significant difference as regards hemoglobin (HGB) between IDA (9.4±1.12 mg/dl) and BTT (8.2±1.67 mg/dl), being less in patients with BTT, with a P-value of 0.000. This aligns with *Matos et al.'s* study, which revealed a statistically significant difference between IDA and BTT in HGB level, being lower in IDA with P-value <0.0001 ***(17).*** Moreover, *Matos et al.'s* study reported a decreased hemoglobin concentration in thalassemia trait ***(18).***

Reticulocyte percentage was significantly higher in BTT(2.58±1.4 mg/dl)

than IDA (.79±.36 mg/dl)with a P-value of 0.000. Likewise, *Ekram and colleagues* also stated a high reticulocyte count in BTT compared to IDA***(19).*** High reticulocyte frequency revealed a hyperactive bone marrow, a characteristic feature of hemolytic anemia.

Moreover, MCV was significantly lower in BTT children than in those with IDA (P-value 0.000). This goes on the run with numerous previous studies***(11, 20).*** This can be explained as microcytosis associated with IDA being lower than that related to the thalassemia trait, so the disturbance in hemoglobin synthesis is slighter than in the thalassemia trait ***(18).*** Similarly, MCH was also significantly lower in the BTT group (median range 19.5 (16.5-23)) compared to the IDA group(median range 22.4 (18-24)) with a P-value of 0.008. This aligns with *Matos et al.,* where there was a statistically significant difference between IDA and BTT in MCH with a P-value.001 ***(17),*** and another study by *Ayman and colleagues* reported the same difference with the P-value. 002 ***(13).***

The red cell distribution width (RDW) test measures the red blood cell (RBC) volume variation range. There was a statistically significant difference between IDA 15.25(14.5-18) and BTT 13.1(12-16.5), with IDA being higher in IDA with a P-value of 0.000. The same was reported by *Jameel's study* in which there was a statistically significant difference between IDA and BTT in RDW, with P-value <.001 being higher in IDA ***(11).*** This contrasts with *Ayman's study*, which found no statistically significant difference between IDA and BTT regarding RDW, with a P-value of 431 ***(13).***

RDWI is calculated using the formula (RDWI=MCV XRDW/RBC). This study showed a statistically significant difference regarding RDWI between IDA and BTT. RDWI was lower in BTT with a P-value of 0.000.

The present study revealed that RDWI had better sensitivity and specificity (83.33%, 80% respectively for IDA and 80%, 83.33% respectively for BTT) than RDW (80%, 66.67% respectively for IDA, and 66.67%, 80% respectively for BTT). These results were very close to a study conducted by *Jameel et al.,* which revealed that the sensitivity and specificity of RDW in detecting βTT were not significant, where RDWI was a good discriminator between them. Its sensitivity and specificity were more than 80% in detecting βTT and IDA ***(11).***

Regarding serum iron, our study revealed a statistically significant difference between IDA and BTT, with a P-value of 0.000 being lower in IDA. Also, our study reported a significant difference between BTT and IDA regarding serum ferritin level (with P-value=0.000) and TIBC with P-value =0.000. TIBC was higher in IDA children.

Conclusions

Red cell distribution index (RDWI) is more reliable than RDW for differentiation between IDA and BTT, and it is an easy, cheap, and more accessible parameter without being time-consuming and more valuable in initial screening.

Conflict of interest

The authors declare that there was no conflict of interest regarding the publication of this paper.

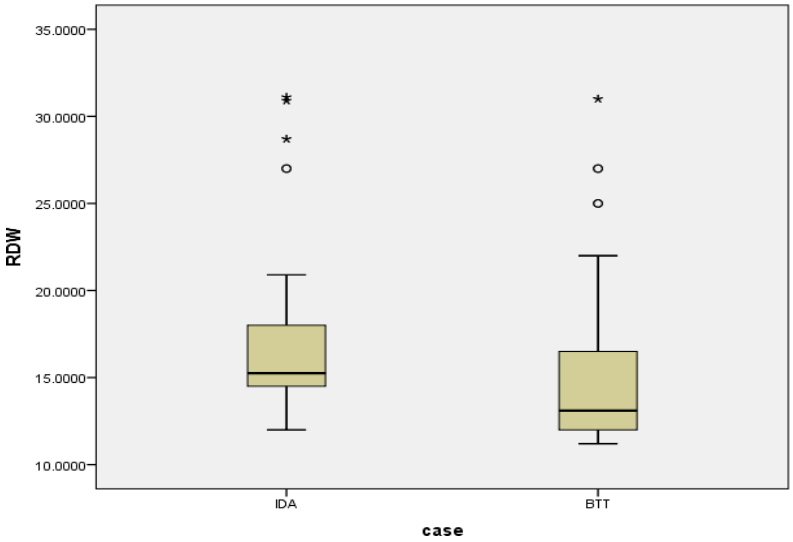

Figure (1): RDW in the two studied groups

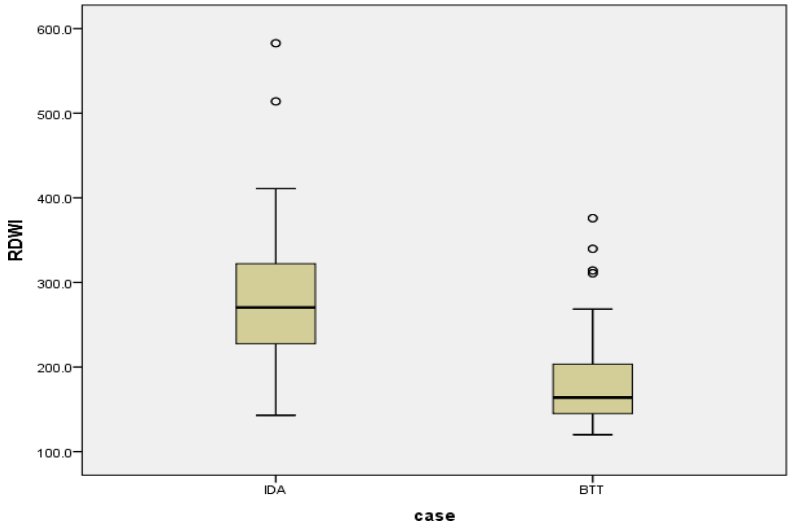

Figure (2): RDWI in the two studied groups

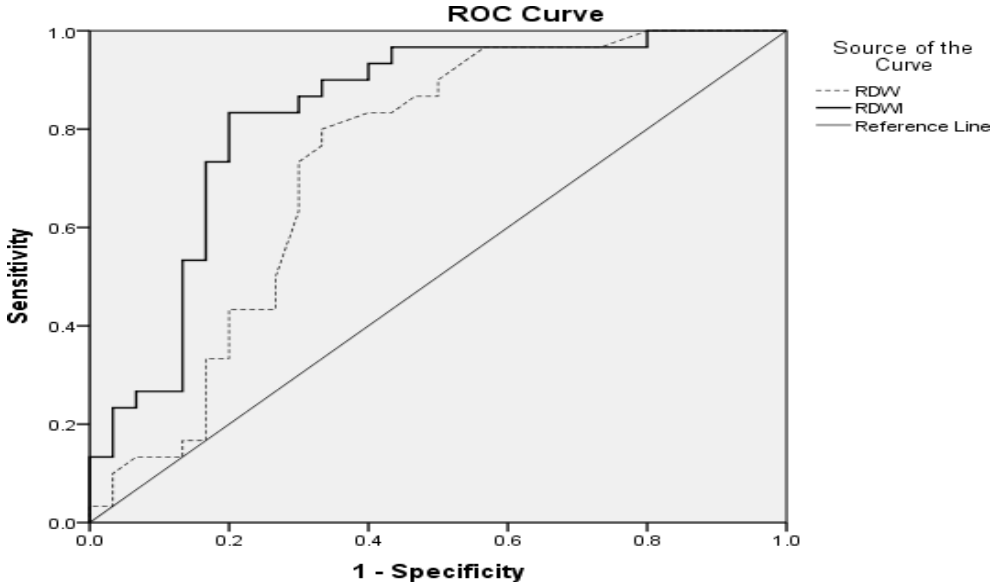

Figure (3): The ROC Curve displays the Diagnostic Accuracy of RDW and RDWI to discriminate between IDA and BTT in the studied patients

Figure (3) shows that RDW could significantly discriminate between IDA and BTT with an area under the curve equal to 0.732 (P-value<0.001), and RDWI could significantly discriminate between IDA and BTT with an area under the curve equal to 0.83 (P-value < 0.001).

Table (1): Gender and age distribution in the two studied groups:

		IDA group No.(%)	BTT group No.(%)	p-value
Gender	**Male =86pts**	46(76.7%)	40(66.7%)	0.224
	Female=34	14(23.3%)	20(33.3%)	

	IDA group Mean ± SD	BTT group Mean ± SD	p-value
Age	5.28±3.1	6.46±3.87	0.065

Table (2): CBC Parameters in the study groups:

	IDA group	BTT group	p-value
	Mean ± SD	**Mean ± SD**	
HGB g/dl	9.4 ± 1.12	8.2 ± 1.67	0.000*
RETICS %	0.79± 0.36	2.58 ± 1.4	0.000*
	Median (IQR)	**Median (IQR)**	
MCV (fi)	67(65.3-70.5)	58.5(55-60.9)	0.000*
MCH(pg)	22.4(18-24)	19.5(16.5-23)	0.008*
MCHC(g/dl)	33.95(33.6-34.6)	32(30.5-33.8)	0.000*
RBCs×10^6/ul	4(3.7-4.6)	4.8(4.5-5)	0.000*
RDW	15.25(14.5-18)	13.1(12-16.5)	0.000*
WBCs×10^3/ul	11.6(5.6-16.5)	7.9(6.5-11.6)	0.171
RDWI	270.4(227.7-322.1)	163.9(145-203.5)	0.000*

Table (3): Iron study in the two studied groups

	IDA group Median (IQR)	BTT group Median (IQR)	p-value
S. iron mcg/dl	30(20-32)	100(70-110)	0.000*
TIBC	480(466-510)	300(280-338)	0.000*
S. ferritin ng/ml	9.5(8-11.2)	131.5(90-168)	0.000*

Table (4): Best cut-off points of RDW and RDWI to discriminate between IDA and BTT in the studied patients

	Cut off	Case	Sensitivity %	Specificity %	PPV %	NPV %	Youden's index
RDW	> 14	IDA	80	66.67	70.6	76.9	46.67
	< 14	BTT	66.67	80	76.9	70.6	
RDWI	> 220	IDA	83.33	80	80.6	82.8	63.33
	< 220	BTT	80	83.33	82.8	80.6	

PPV: positive predictive value. NPV: negative predictive value (NPV)

References:

1. Sankar VH, Arya V, Tewari D, Gupta UR, Pradhan M, Agarwal S. Genotyping of alpha-thalassemia in microcytic hypochromic anemia patients from North India. J Appl Genet. 2006;47(4):391-5.
2. Vehapoglu A, Ozgurhan G, Demir AD, Uzuner S, Nursoy MA, Turkmen S, et al. Hematological indices for differential diagnosis of beta thalassemia trait and iron deficiency anemia. Anemia. 2014;2014:576738.
3. Tripathi N, Soni JP, Sharma PK, Verma M. Role of haemogram parameters and RBC indices in screening and diagnosis of beta-thalassemia trait in microcytic, hypochromic Indian children. Int J Hematol Disord. 2015;2(2):43-6.
4. Bordbar E, Taghipour M, Zucconi BE. Reliability of different RBC indices and formulas in discriminating between β-thalassemia minor and other microcytic hypochromic cases. Mediterr J Hematol Infect Dis. 2015;7(1):e2015035.
5. McClure S, Custer E, Bessman JD. Improved detection of early iron deficiency in nonanemic subjects. JAMA. 1985;253(7):1021-3.
6. Plengsuree S, Punyamung M, Yanola J, Nanta S, Jaiping K, Maneewong K, et al. Red cell indices and formulas used in differentiation of β-thalassemia trait
7. from iron deficiency in Thai adults. Hemoglobin. 2015;39(4):235-9.
8. Park Y, Yamauchi T, Choi W, Dasari R, Feld MS. Spectroscopic phase microscopy for quantifying hemoglobin concentrations in intact red blood cells. Opt Lett. 2009;34(23):3668-70.
9. Jayabose S, Giamelli J, LevondogluTugal O, Sandoval C, Ozkaynak F, Visintainer P. Differentiating iron deficiency anemia from thalassemia minor by using an RDW-based index. J Pediatr Hematol Oncol. 1999;21(4):314.
10. Bhaumik S, Patel S. Nutritional Anaemia in Adolescent Girls in India-An Insight. SciFed J Anal Biochem. 2018;1(1).
11. Mukhopadhyay D, Saha K, Sengupta M, Mitra S, Datta C, Mitra PK. Role of discrimination indices in screening of beta-thalassemia trait in West Bengal, India: An institutional experience on 10,407 subjects. Saudi J Health Sci. 2015;4(3):151-5.
12. Jameel T, Baig M, Ahmed I, Hussain MB, Alkhamaly MBD. Differentiation of beta thalassemia trait from iron deficiency anemia by hematological indices. Pak J Med Sci. 2017;33(3):665-9.
13. Aslan D, Gümrük F, Gürgey A, Altay C. Importance of RDW value in differential

diagnosis of hypochrome anemias. Am J Hematol. 2002;69(1):31-3.
14. Ayman S, Ahmed A, Abou-Taleb A. Assessment of new indices to discriminate between iron deficiency anemia and thalassemia trait in Sohag University. Sohag Med J. 2019;23(3):49-62.
15. Hoffmann JJ, Urrechaga E, Aguirre U. Discriminant indices for distinguishing thalassemia and iron deficiency in patients with microcytic anemia: a reply. Clin Chem Lab Med. 2016;54(4):e107-e8.
16. Warghade S, Britto J, Haryan R, Dalvi T, Bendre R, Chheda P, et al. Prevalence of hemoglobin variants and hemoglobinopathies using cation-exchange high-performance liquid chromatography in central reference laboratory of India: A report of 65779 cases. J Lab Physicians. 2018;10(1):73-9.
17. Ismail M, Patel NG. Evaluation of the Diagnostic Accuracy of Twelve Discrimination Indices for Differentiating β-thalassemia Trait from Iron Deficiency Anemia. Indian J Public Health Res Dev. 2016;7(1):104-9.
18. Matos JF, Dusse LMS, Stubbert RVB, Ferreira MR, Coura-Vital W, Fernandes APSM, et al. Comparison of discriminative indices for iron deficiency anemia and β thalassemia trait in a Brazilian population. Hematology. 2013;18(3):169-74.
19. Matos JF, Borges KB, Fernandes APS, Faria JR, Carvalho MDG. RDW as differential parameter between microcytic anemias in "pure" and concomitant forms. J Bras Patol Med Lab. 2015;51(1):22-7.
20. Ekram AR, Hanan SA, Mahmoud AA, Osama M. The Diagnostic Performance of Red Cell Distribution Width and Mentzer Index for Discrimination between Iron Deficiency Anemia and Beta Thalassemia Trait. Med J Cairo Univ. 2018;86(December):3979-88.
21. Jahangiri M, Rahim F, Malehi AS. Diagnostic performance of hematological discrimination indices to discriminate between βeta thalassemia trait and iron deficiency anemia and using cluster analysis: Introducing two new indices tested in Iranian population. Sci Rep. 2019;9(1):18610.