

ORIGINAL ARTICLE

Cut off Value of Red Cell Distribution Width (RDW) in Screening and Diagnosis of Iron Deficiency Anemia and β Thalassemia Trait

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Abstract

Background: The most concerning consequences of iron deficiency in children are the alterations of cognitive, motor, and behavioral performance. Persistent neurocognitive changes despite iron repletion have increased the importance of prevention and early detection of iron deficiency.

Objectives: To evaluate the cut off value of red cell distribution width (RDW) in the screening and diagnosis of iron deficiency anemia (IDA) and β thalassemia trait (BTT).

Methods: It is a cross sectional study performed at Dhaka Shishu (Children) Hospital from July 2006 to July 2008. Total 52 patients, age ranged from 1-12 years were included in the study. Among them 27 cases were IDA and 25 cases were BTT. Complete blood count (CBC), serum ferritin and hemoglobin (HB) electrophoresis were measured. The sensitivity, specificity, positive and negative predictive value of differential and cutoff value for RDW discrimination index in differentiation between IDA and BTT were performed.

Results: Age ranged from 1-12 years. In IDA group male were found 16(59%), female were 11(41%) and in BTT group male were 15(60%), female were 10(40%). The Hb% value in IDA group was significantly ($p < 0.0001$) lower than value for BTT. An elevation of RBC distribution width (>14.6) in IDA had a sensitivity of 81%, specificity of 84%, positive predictive value (PPV) of 85% and negative predictive value (NPV) of 81%; value of RBC distribution width (<14.6) in BTT had a sensitivity of 84%, specificity of 81%, positive predictive value (PPV) of 81% and negative predictive value (NPV) of 85%. An elevation of RBC distribution width (>16) had a sensitivity of 67%, specificity of 92%, and positive predictive value (PPV) of 90% in distinguishing iron deficiency anemia from thalassemia trait. Moreover, eleven (11) of 27 patients with iron deficiency had RBC distribution width values greater than 18 compared to one (1) of the patients with thalassemia trait. An elevation of RBC distribution width ≥ 18 specificity of 96% and positive predictive value (PPV) of 92%.

Conclusion: The result of the study, in patients with microcytic hypochromic anemia, RDW value $\geq 14.6\%$ and Hb level $< 10\text{gm/dl}$, iron deficiency is the most likely diagnosis. RDW value (>16) is a reliable diagnostic tool in differentiation between IDA and BTT. Red cell distribution width (RDW) above 16% is the best index of IDA.

Keywords: Anemia, iron deficiency anemia (IDA), β thalassemia trait (β T), RBC distribution width (RDW).

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Introduction

Iron deficiency anemia is the most prevalent nutritional problem in the world today.¹ It was estimated that the number of anemic people worldwide were two billion and that approximately 50% of them were due to iron deficiency. Because anemia is the most common indicator used to screen for iron deficiency, the terms anemia, iron deficiency, iron deficiency anemia are sometimes used interchangeably.²

In Bangladesh prevalence of anemia among children aged 6-59 months (preschool children) is 47%. The highest proportion (76%) of anemic children belonged to the 6-11-months of age group.³

During the past decade, the possible association between iron deficiency, with or without anemia, and impaired cognitive and psychomotor development has been the subject of much concern. This concern has led to establishing extensive intervention programmes to prevent iron deficiency in many countries.⁴ The brain's sensitivity to iron deficiency is mitigated by the severity and timing of the deprivation and the adverse effects of iron deficiency may or may not be reversible.⁴⁻⁶

Iron deficiency (ID) is by far the most common cause of anemia in general and of microcytic hypochromic anemia in particular. The most common scenario is the need to distinguish iron deficiency anemia (IDA) from β thalassemia trait (β TT).⁷ The thalassemia probably constitutes the world's largest gene disorder and it is one of the commonest inherited diseases in Bangladesh.⁸ A conservative World Health report has estimated that 3 percent are carriers of β thalassemia and 4 percent are carriers of Hb-E disease in Bangladesh.⁹ Patients of \hat{a} thalassemia trait (heterozygous state of thalassemia) are near normal except that they are anemic. The patients of β thalassemia trait does not need iron therapy unless there is iron deficiency. Genetic counseling is the effective way to prevent the population from thalassemia; the population at risk needs to be identified. Fortunately iron deficiency anemia (IDA) is treatable. Health care providers have long subscribed to a program of screening for and treating iron deficiency to avoid the consequences of this disorder.¹⁰⁻¹²

Several approaches are used to assess the iron status of an individual or of a population. Serum ferritin concentration is the most powerful test for ID.¹³ A

serum ferritin concentration of <12 μ g/dl is diagnostic of ID.¹⁴ Additional investigations may also be needed for detection of iron deficiency anemia, such as estimation of serum transferrin receptor, total iron binding capacity (TIBC), serum iron level transferrin saturation & free erythrocyte protoporphyrin (EPP).

It would be impractical, however, to use initially the whole battery of above investigations and simpler approaches for population and individual studies have been suggested.¹⁵ Again, to rule out \hat{a} thalassemia trait, hemoglobin electrophoresis is needed. All these tests are expensive and time consuming and constitute a significant burden on public health economy in countries with high incidence of microcytic hypochromic anemias. Developing countries have high prevalence of iron deficiency anemia, but facilities to diagnose and treat them are limited.¹⁶

Iron deficiency anemia (IDA) and thalassemia trait (TT) are the two most common forms of microcytic hypochromic anemia. Therefore there is a need for a simple, low cost rapid and reliable, common routine investigation, which can be used for screening and can easily differentiate between \hat{a} thalassemia trait and iron deficiency anemia. In order to distinguish between the two, discrimination indices calculated from red blood cell (RBC) indices are used.^{17,18} These indices are derived from several simple red blood cell (RBC) indices, like RBC count, mean corpuscular volume (MCV), and Red cell distribution width (RDW), which are provided by electronic cell counters.

Thalassemia minor and iron deficiency anemia are both microcytic and hypochromic anemia, overlap in MCV and MCH. The RDW aids in differentiating anemia that have similar indices. Measures of anisocytosis derived from erythrocyte volume distribution have been advocated for distinguishing iron deficiency from thalassemia minor.¹⁹⁻²¹ Red-cell distribution width (RDW) allows discrimination of iron deficiency anemia and thalassemia trait.²¹ Kook et al²² postulated that difference in size and range variation might be diagnostically useful.

The purpose of the study is to see the sensitivity, specificity, positive predictive value and negative predictive value of the test of significance and is to evaluate validity of RDW in the screening and diagnosis of iron deficiency anemia and to differentiate between iron deficiency anemias (IDA)

from β thalassemia trait (β TT). This study might serve as model of management strategy for treating iron deficiency to avoid the consequences of this disorder.

Materials and Methods

This hospital based cross sectional study was conducted during the period of July 2006 to July 2008. Total 52 patients of 1-12 years age with full verbal explanation to their parents/attendants were included in this study. IDA patients were in group-1 and BTT patients were in group-2, cases were selected from Out Patient Department (OPD) and Thalassemia Center (anemic sibs of thalassemia patients) respectively. Iron deficiency anemia (IDA) cases with Hb value <8.7 gm/dl were excluded because these cases were confused with β thalassemia trait (β TT) in practice. The subjects who had been on any hematinic in last 2 months or received blood transfusion in the past 3 months, acute febrile illness >5 days, patients who were chronically ill and severely malnourished,, patients with active diseases and those who needed hospital admission were excluded from the study. For each patient, a detail history which included age, gender, family history, consanguinity, socioeconomic status, medical history, diet, use of medications, anemia related symptoms (Pallor, Fatigue, Exercise intolerance, Tachycardia, Palpitation, Irritability & Anorexia) was taken.

In subjects of the group-1, Serum ferritin <12 ng/ml was diagnosed to have IDA¹⁴ and of group-2, Hemoglobin (Hb)A2 ≥ 3.5 % was diagnosed to have β thalassemia trait. The normal value for RDW is $13.4 \pm 1.2\%$ (mean \pm 2SD) and the upper limit of normal is 14.6%.^{18,19} A subject was considered to have an elevated red cell distribution width if the value exceeded >14.6 %.¹⁹

Complete blood count (CBC) included values of hemoglobin (Hb), RBC count, mean red cell volume (MCV), mean red cell hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and red cell distribution width (RDW). Hematological analyses were measured in all the selected cases with Mythic 18 continuous flow automated analyzer.

Serum ferritin were being assessed using Dimension Rx L Max Biochemistry fully auto analyzer (dade Behring, USA products).Hb electrophoresis was performed on cellulose agar gel at alkaline pH by

Helena 5 automated machine. Data and results were presented in the form of tables and figures. Analysis was done by employing statistical package for social science (SPSS Version 12.0) software package. Student's 't' test in continuous variable & chi-square test in categorical variable were calculated. In addition descriptive statistics such as frequency tabulation, mean, median, standard deviation (SD) and test of significance were calculated. The sensitivity, specificity, positive predictive values and negative predictive values of RDW and RBC count were calculated. Statistically significant results were those with values of $p \leq 0.05$.

Results

Age ranged from 1-12 years. In IDA group male were found 16(59%), female were 11(41%) and in BTT group male were 15(60%), female were 10(40%) (Fig.-1).

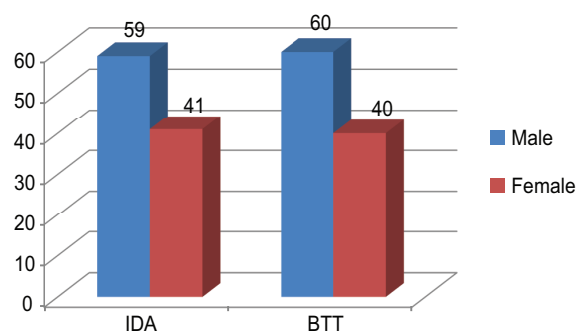


Fig.-1 Gender distribution in both groups

In this study, haematological parameters (Hb%, RBC count, MCV, MCH, MCHC and RDW) of IDA group and BTT group was reviewed and it was found that Hb%, RBC count and RDW was significantly different between the two groups. The mean Hb% value in IDA group was $9.28 (\pm 0.68)$; the mean Hb% value for BTT was 10.26 ± 0.34 . The Hb% value in IDA group was significantly ($p < 0.0001$) lower than the Hb% for BTT. The mean value of MCV was 69.56 ± 4.44 fl in IDA group, in BTT group the mean value of MCV was 67.65 ± 3.79 fl; mean value of MCH in IDA group was 19.40 ± 2.49 pg and in BTT group was 19.28 ± 1.90 pg and the mean value of MCHC in IDA group 27.93 ± 1.15 gm/dl in BTT group was 28.18 ± 1.05 gm/dl. All the above patients of two groups had microcytic hypochromia, three indices MCV, MCH and MCHC overlap in two groups, and there was no significant difference in MCV, MCH and MCHC of two groups.

The mean RDW value for IDA was 16.75 (± 2.37), the mean RDW value for BTT was 13.43 (± 1.50). The RDW value for IDA was significantly ($p < 0.0001$) greater than value for BTT. Contrary, The RBC count for BTT was significantly ($p < 0.0001$) greater than value for IDA. The mean RBC count for IDA was 4.41 (± 0.41), the mean RBC count for BTT were 5.20 (± 0.35 /mm³). From this result, the study revealed that to differentiate between iron deficiency anemia (IDA) & beta thalassemia trait (BTT) hemoglobin (Hb) level, red cell distribution width (RDW) value and red blood cell (RBC) count might play pivot role in common practice (Table I).

The numbers of patients in each group with an elevated RBC distribution width. Twenty two (22) of

27 patients with iron deficiency had RBC distribution width values greater than 14.6, compared to four (4) of 25 patients with thalassemia trait, and twenty one (21) of 25 patients with thalassemia trait had RBC distribution width values lower than 14.6, compared to five (5) of 27 patients with iron deficiency. The total number of correctly identified patients by using indices RDW is 43(83%). So this index helps us in distinguishing iron deficiency anemia from thalassemia trait. In this study it was found that, eleven (11) of 27 patients with iron deficiency had RBC distribution width values greater than 18 compared to one (1) of the patients with thalassemia trait (Table II).

Table I

Difference of Haematological parameters; Hb%, RBC count, MCV, MCH, MCHC and RDW of IDA group (n=27) and BTT group (n=25)

Haematological parameters	IDA (n=27) Mean (\pm SD)	BTT (n=25) Mean (\pm SD)	P value
Hb%(gm/dl)	9.28(\pm 0.68)	10.26 (\pm 0.34)	<0.001
RBC X 10 ¹² /L	04.41(\pm 0.41)	05.20(\pm 0.35)	<0.001
MCV(fl)	69.56(\pm 04.44)	67.65 (\pm 3.79)	0.10
MCH (pg)	19.40(\pm 02.49)	19.28(\pm 01.90)	0.97
MCHC(gm/dl)	27.93(\pm 01.05)	28.18 (\pm 01.15)	0.40
RDW	16.75(\pm 2.37)	13. 43(\pm 1.50)	<0.001

Table II

The differential value of RDW for differentiation between IDA and BTT and correctly identified number of the patients by using these indices in each group

Differential value	IDA (n=27)	BTT (n=25)	Total number of Correctly diagnosed Patients (n= 52)	Percentage correctly Identified patients (%)
RDW (%)				
IDA >14.6	22*	4	43(22+21)	83%
BTT <14.6	5	21 *		

*True positives. IDA- iron deficiency anemia; β TT- beta thalassemia trait; RBC- red blood cells; RDW- red blood cell distribution width

An elevation of RBC distribution width (>14.6) in IDA had a sensitivity of 81%, specificity of 84%, positive predictive value (PPV) of 85% and negative predictive value (NPV) of 81%; value of RBC distribution width (<14.6) in BTT had a sensitivity of 84%, specificity of 81%, positive predictive value (PPV) of 81% and negative predictive value (NPV) of 85% (Table III).

Table III

Sensitivity, specificity, positive and negative predictive value of discrimination index RDW in differentiation between BTT and IDA

Indices	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
RDW (%)				
IDA >14.6	81%	84%	85%	81%
BTT <14.6	84%	81%	81%	85%

PPV, positive predictive value; NPV, negative predictive value; positive predictive value: true positive/(true positive + false positive); negative predictive value: (true negative/true negative + false negative)

In this study patients with microcytic anemias, RBC distribution width was elevated in most patients with iron deficiency anemia. Eighteen (18) of 27 patients with iron deficiency had RBC distribution width values greater than 16, compared to two (2) of 25 patients with thalassemia trait. Above table also shows that twenty three (23) of 25 patients with thalassemia trait had RBC distribution width values lower than 16, compared to nine (9) of 27 patients with iron deficiency (Table IV).

Table IV

The differential value of RDW, when cutoff value for RDW >16 in differentiation between IDA and BTT.

Differential value	IDA (n=27)	BTT (n=25)
RDW (%)		
IDA >16	18*	2
BTT <16	9	23 *

*True positives. IDA, iron deficiency anemia; β Tt, beta thalassemia trait; RBC, red blood cells; RDW, red blood cell distribution width

An elevation of RBC distribution width (>16) had a sensitivity of 67%, specificity of 92%, and positive predictive value (PPV) of 90% indistinguishing iron deficiency anemia from thalassemia trait. Moreover, eleven (11) of 27 patients with iron deficiency had RBC distribution width values greater than 18 compared to one (1) of the patients with thalassemia trait. An elevation of RBC distribution width ≥ 18 specificity of 96% and positive predictive value (PPV) of 92%. The table had shown validity of RDW that predict presence of IDA and in distinguishing iron deficiency anemia from thalassemia trait (Table V).

Table V

Sensitivity, specificity, positive and negative predictive value of RDW discrimination index in differentiation between BTT and IDA, when cutoff value for RDW >16

Indices	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
RDW (%)				
IDA >16	67 %	92 %	90 %	72 %
BTT <16	92 %	67 %	72 %	90 %

PPV, positive predictive value; NPV, negative predictive value

Discussion

Iron deficiency (ID) is by far the most common cause of anemia in general and of microcytic hypochromic anemia in particular. It is important to make a timely and accurate diagnosis and initiate an early intervention to reduce the negative impact of anemia. Screening for BTT is of increasing importance in genetic counseling. However, differentiating BTT from IDA is warranted because the thalassemia heterozygote should not be given iron in a IV route to normalize MCV.²³

Decreased levels of serum iron (SI), transferrin saturation (TS) and ferritin with increased levels of total iron binding capacity (TIBC) are the main diagnostic criteria for IDA.^{17,18} The presence of characteristic red blood cell microcytosis and elevated levels of HbA2 establish the diagnosis of BTT. Showing body iron status or measuring HbA2 is time-consuming and requires laboratory staff and also not possible at every centers.

Less time-consuming methods are based on the calculation of discrimination indices from red blood cell indices obtained during routine complete blood count. On the basis of this approach, since 1973 some discrimination indices such as Mentzer's index (MI), RBC count, RDW, and RDWI are reported for differentiation between thalassemia trait (TT) and iron deficiency anemia (IDA).²⁴ Several studies have been done throughout the world to see sensitivity & specificity of most reliable RBC indices.

Though there are many similarities of red cell indices in IDA and BTT but RDW value emerged as an important parameter to differentiate iron deficiency anemia (IDA) from β thalassemia trait (β TT).^{20,21,25,26}

There are few publications regarding these topics in pediatrics. An assessment of the value of RDW in the evaluation of pediatric microcytic anemias is the purpose of this study.

The study consists of IDA & BTT subjects; IDA was diagnosed as serum ferritin <12ng/ml and BTT was diagnosed as hemoglobin (Hb) A2 \geq 3.5%. In this study, 27 cases of IDA and 25 cases of BTT were the subjects of the study.

Regarding mean weight, height and the mean monthly income of both group showed there was no significant difference of these parameters in the two (2) groups. In present study, the mean age was 5.48(\pm 2.83) years in IDA group & in BTT group it was 5.76(\pm 3.03) years. There was no significant difference between IDA & BTT groups in respect of age ($P > 0.05$).

Consanguinity was found only in BTT group with a large percentage 16(64%) compare to IDA group (0%).

The present study revealed that the mean hemoglobin level was 9.28(\pm 0.68) gm/dl in IDA group & in BTT it was 10.26 (\pm 0.34). Statistically significant differences were found between iron deficiencies with b-thalassaemia trait ($P < 0.001$).

The mean haemoglobin level in the b- thalassaemia trait was higher than IDA, it was observed by Demir et al¹². they were found mean Hb% in BTT 12.41(\pm 1.60) & in IDA it was 9.35(\pm 0.81) gm/dl. Madan et al²⁷ and England et al²⁸ obtained also similar results, mean Hb concentration was significantly higher in traits as compared to iron deficient subjects ($p < 0.0001$).

In this study, the mean value of MCV was 69.56 \pm 04.44 fl in IDA group and in BTT group it was 67.65 \pm 3.79 fl; mean value of MCH in IDA group was 19.40 \pm 02.49 pg and in BTT 19.28 \pm 01.90 pg and the mean value of MCHC was in IDA 27.93 \pm 01.05 gm/dl and in BTT group 28.18 \pm 01.15 gm/dl. There was no significant difference of these haematological parameters in IDA and BTT groups. MCV and MCH in beta thalassemia trait (BTT) had lower levels than of iron deficiency anemia. Some study also showed that, MCV and MCH in b- thalassemia trait had higher levels than that of iron deficiency anaemia.²⁹⁻³⁰

The present study showed that the RBC count in b-thalassaemia trait is significantly higher than that of iron deficiency anemia ($p < 0.0001$). The mean RBC counts was 05.20 \pm 0.35 x 10¹²/L in BTT & the mean RBC count in IDA was 04.41 \pm 0.41 x 10¹²/L. England et al¹⁹ and Demir et al²⁴ found that b- thalassaemia trait had significantly higher number of red cells than that of iron deficiency anaemia.

In this study, the mean RDW value for IDA was 16.75 \pm 2.37 and the mean RDW value for BTT was 13.43 \pm 1.50. The RDW values for IDA are significantly ($p < 0.0001$) greater than value for BTT.

The findings of present study was similar with the findings of Robert et al³¹, where the mean RDW was in IDA 19.45 \pm 4.02; and the mean RDW in BTT was 14.91 \pm 1.13. Similar finding also were found by Bessman et al²¹ and Demir et al²⁴ in their studies, the mean Red cell distribution width (RDW) in iron deficiency anaemia (IDA) was significantly higher than the mean RDW in beta thalassemia trait (BTT).

Besides calculating the sensitivity, specificity, positive and negative predictive value of RDW and RBC count in differentiation between BTT and IDA, total number of correctly identified patients also calculated in this study.

In the present study the total number of correctly identified patients by using RDW value is 43(83%) and RBC count is 43(83%) in each group. Similar finding regarding RBC count conforms to the study of Demir et al²⁴ They found, total number of correctly diagnosed patient by RBC was 90%, but by RDW it was only 59%.

This study revealed that, an elevation of RBC distribution width (RDW) (>14.6) in IDA had sensitivity of 81% and specificity of 84% to differentiate iron deficiency anemia from thalassemia

trait. The positive predictive value (PPV) of RDW >14.6 in cases of IDA were 85%. Robert et al³¹ found an elevation of RBC distribution width had a sensitivity of 86%, specificity of 57% to distinguishing iron deficiency anemia from thalassemia trait. Viswanath et al²⁵ detected role of red cell distribution width in the diagnosis of iron deficiency anemia as RDW had a higher sensitivity than peripheral smear (PS). They also found RDW was suggestive of iron deficiency in 100%, 82.05% and 100 % of patient with mild, moderate and severe anemia respectively.

Regarding RBC count, in this study it was found that an elevation of RBC ($>5 \times 10^{12}/L$) in BTT had a sensitivity of 83% and specificity of 86%. The positive predictive value (PPV) of RBC count $>5 \times 10^{12}/L$ in cases of BTT was 83%. Demir et al²⁴ was found ninety percent of the children patients were correctly identified with RBC count.

In this study it was found that, the positive predictive value (PPV) of RDW >14.6 in cases of IDA and the positive predictive value (PPV) of RBC count $>5 \times 10^{12}/L$ in cases of BTT were highest as follows: 85% and 83% respectively.

Robert WN found that none of the patients with thalassemia trait had RBC distribution width values greater than 20, elevation of RBC distribution width to greater than 20 was seen exclusively in iron deficiency.³¹

This study showed that when cutoff value for RDW >16 in differentiation between IDA and BTT had Sensitivity 67%, Specificity 92% and positive predictive value (PPV) 90%; and an elevation of RBC distribution width had e"18 specificity of 96% and positive predictive value (PPV) of 92%. Similar study was done by Melo et al³² they mentioned that, RDW above 16% was the best index of IDA, with sensitivity 69.2% and specificity 80.7% and Laso et al³³ found When 18 was taken as cutoff value for RDW, its positive predictive value was very high in iron deficiency (95%).

In this study, it was found that, the positive predictive value (PPV) of RDW>14.6 are the highest (85%) than other RBC indices. When cutoff value for RDW e"16 % and e"18% to diagnosed iron deficiency anemia (IDA), the positive predictive value (PPV) was 90% and 92% respectively. In such a way, RDW value might be reliable diagnostic tool for differentiation between IDA and BTT.

Conclusion

From this study, it may be concluded that Haemoglobin level, RBC distribution width (RDW) and RBC count appears to be perfect and useful device for the management of pediatric microcytic hypochromic anemia. In patients with microcytic hypochromic anemia, RDW value $\geq 14.6\%$ and Hb level $< 10\text{gm/dl}$, iron deficiency is the most likely diagnosis. RDW value (>16) is a reliable diagnostic tool in differentiation between IDA and BTT. Red cell distribution width (RDW) above 16% is the best index of IDA.

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