Annals of International Medical and Dental Research
E-ISSN: 2395-2822 | P-ISSN: 2395-2814
Vol-8, Issue-3 | May-June 2022
DOI: 10.53339/aimdr.2022.8.3.14
Page no- 107-113 | Section- Research Article (Pathology)

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

Biva Rani Mondal[1]*, Mohammad Kamrul Hossain[2], Alamgir Ahmed[3], Lotifur Rahman[4], Kishor Chandra Pal[5]

[1]Assistant Professor, Department of Clinical Pathology, Bangladesh Shishu Hospital and Institute, Dhaka, Bangladesh. Email: bivadsh@gmail.com, Orcid ID: 0000-0001-6156-283X
[2]Assistant Professor, Department of Anesthesia and ICU, Cumilla Medical College, Bangladesh. Email: kamrulhossain32@gmail.com, Orcid ID: 0000-0003-1377-6759
[3]Assistant Professor, Department of Clinical Pathology, Bangladesh Shishu Hospital and Institute, Dhaka, Bangladesh. Email: Alamgir587@gmail.com, Orcid ID: 0000-0002-0479-4181
[4]Senior Medical Technologist, Department of Clinical Pathology, Bangladesh Shishu Hospital and Institute, Dhaka, Bangladesh. Email: latifur62@gmail.com, Orcid ID: 0000-0001-9565-6376
[5]Senior Medical Technologist, Department of Clinical Pathology, Bangladesh Shishu Hospital and Institute, Dhaka, Bangladesh. Email: kishorchpal@gmail.com, Orcid ID: 0000-0002-5942-2537

*Corresponding author

Received: 07 February 2022
Revised: 24 March 2022
Accepted: 03 April 2022
Published: 22 April 2022

Abstract

Background: Iron deficiency anemia (IDA) is one of the common nutritional disorders in the world. In the subcontinent Microcytic hypochromic anemia is usually caused by beta thalassemia trait (BTT) and iron deficiency anemia (IDA). IDA may be confused with BTT. It is important to distinguish between the above conditions to avoid unnecessary iron therapy in thalassemia carriers. Red cell distribution width index (RDWI) are a simple, easy, and cost effective method to get a primary and valuable information regarding the diagnosis of IDA and BTT. **Objective:** To assess the predictive value of Red cell distribution width index (RDWI)) for differentiation of Iron Deficiency Anaemia and Beta Thalassaemia Trait. **Material & Methods:** The study was a cross-sectional descriptive study which was conducted in Department of Clinical Pathology, Bangladesh Shishu Hospital and Institute, Dhaka, Bangladesh. Over a period of April 2019 to September 2020. The newly clinically diagnosed cases of BTT and IDA were selected for this study. The sample size was 110. Among them 46 cases were identified as BTT and 64 were IDA. Data were analysed using a computer programme SPSS 25.0 version. **Results:** Total 110 respondents were included in the study. Among them 46.4% were female and 53.6% were male. About 72.72 % of respondents were aged 1 to 10 years old, while 10% were aged 11 to 20 years old and 10% were aged 21 to 30 years old. 4.54 % were between the ages of 31 to 40, as well as those over 40. The average age (SD) was 22.0 ± 32.52. Mean (±SD) age was 22.0 ± 32.52. RDWI had both sensitivity and specificity more than 80% in detection of BTT and IDA. Sensitivity, specificity of RDWI index for detection of BTT was found 81.0%, 83.8%. In case of IDA, sensitivity and specificity was found 83.8% and 81.0% respectively. **Conclusions:** The current study found that multiple discriminants can be used to differentiate between iron deficiency anemia and the Beta Thalassaemia Trait. RDWI could be a better way to tell the difference between BTT and IDA.

Keywords:- RWDI, IDA,BTT.

INTRODUCTION

IDA is one of the common nutritional disorders in the world. In the subcontinent Microcytic hypochromic anemia is usually caused by beta thalassemia trait (BTT) and iron deficiency anemia (IDA).[1] The gene frequency of β -Thalassaemia my varies country to

Annals of International Medical and Dental Research
E-ISSN: 2395-2822 | P-ISSN: 2395-2814
Vol-8, Issue-3 | May-June 2022
DOI: 10.53339/aimdr.2022.8.3.14
Page no- 107-113 | Section- Research Article (Pathology)

country. The highest value was found around the Caspian Sea and Persian Gulf which was more than 10%. But in case of α -thalassemia which is very rare and has no clear report of the prevalence for the country as a whole.[2] To differentiate between beta thalassaemia trait (BTT) and iron deficiency anaemia (IDA), it is very much needed to estimation of HbA2, peripheral blood film, serum ferritin, iron, TIBC (total iron binding capacity) levels and transferrin saturation.[3] Disorders interfering with the formation or rate of production of hemoglobin (Hb) may result in reduction in mean red cell Hb and corpuscular volume (MCV) with resultant hypocromia and microcytosis.[4,5] To ensure the correct diagnosis a full clinical history is very important. Determination of red blood cell (RBC) indices by electronic cell counters represents the first step in the population for mass screening for BTT. And the the second step which is due to exclude IDA, is the Measurement of serum ferritin level in screened subjects with microcytosis and/or hypocromia. The next step is the diagnosis of BTT by quantitation of HbA2 (>3.5%). But these measurements are expensive for public health economy and are not suitable in countries with high prevalence of microcytosis and hypocromia.[6,7,8,9] Many authors have calculated the sensitivity and specificity of these indices to differentiate between IDA and TT.[10] RDW is the first index of the routine blood cell count to become abnormal during the development of iron deficiency.[11] Few discrimination indices calculated from red blood cell count and red blood cell indices are defined and works for rapid discrimination between BTT and IDA. Red cell distribution width index (RDWI) and RDW is considered to be reliable discrimination index in differentiation of BTT and IDA.[7,12,13] The objective of this study is to evaluate the diagnostic value of RDWI for Differentiation of Iron Deficiency Anaemia and Beta Thalassaemia Trait.

MATERIAL AND METHODS

The study was a cross-sectional descriptive study which was conducted in Department of Clinical Pathology, Bangladesh Shishu Hospital and Institute, Dhaka, Bangladesh. Total 110 patient of both sexes with microcytic hypochromic anaemia (46 case with IDA and 64 case with BTT) were selected for this study. A definitive differentional diagnosis between IDA and BTT is based on the results of serum iron levels, serum ferritin and hemoglobin separation. IDA was based on low serum iron and serum ferritin(<30 µg/dl, <15 ng/ml respectivly). Patients already on nutritional supplements, having long standing illness or on medication interfering with micronutrient metabolism (e.g. antiepileptic drugs such as acetazolamide, carbamazepine and clobazam, Aspirin and Antacids containing Magnesium hydroxide) and patients who were diagnosed with or having a family history of haematological disorders other than thalassemia were excluded from the study. The detail of the study was explained to each eligible respondent and consent was taken. 2 ml peripheral venous blood samples were collected under sterile conditions in an EDTA tubes and another 2 ml venous blood collected in the other tubes without anticoagulant for serum separation. Serum was separated within 3 hours of collection. After collection, the data were checked and cleaned, followed by editing, compiling, coding and categorizing

Annals of International Medical and Dental Research
E-ISSN: 2395-2822 | P-ISSN: 2395-2814
Vol-8, Issue-3 | May-June 2022
DOI: 10.53339/aimdr.2022.8.3.14
Page no- 107-113 | Section- Research Article (Pathology)

according to the objectives and variable to detect errors and to maintain consistency, relevancy and quality control. The RBC count, the measurements of Hb, MCV, MCH, MCHC, RDW,RDWI and packed cell volume (PCV/Haematocrit) were obtained by haematology analyser: Sysmex Automated Haematology Analyzer (Model:XN1000) and Mythic -22 using reagent Kits (Diluent, Cleaner, Lytic). Serum iron and ferritin was measured by Enzyme Linked Immunosorbent Assays (ELISA) using reagent kits (IMMULITE and IMMULITE 1000 system). Differentiation between IDA and BTT were done by using red cell distribution width (RDW) and red blood cell distribution width index (RDWI), these were calculated from parameters provided by automated analyzer. Collected data were edited and analyzed according to the objectives and variables by IBM software- Statistical package for Social Science (SPSS 26) version. Ethical clearance was taken from the IRB of the institution.

RESULTS

This study was conducted in Department of Clinical Pathology, Bangladesh Shishu Hospital and Institute, Dhaka, Bangladesh. Total number of the respondents were 110. Among them 46 cases were identified as BTT and 64 were IDA.

[Table 1] shows that 72.72 % of respondents were aged 1 to 10 years old, while 10% were aged 11 to 20 years old and 10% were aged 21 to 30 years old. 4.54 % were between the ages of 31 to 40, as well as those over 40. The average age (SD) was 22.0 ± 32.52.

[Table 2] shows among all the respondents 46.4% were female and 53.6% were male.

[Table 3] shows the Mean±SD haemoglobin level was found 8.9 ±1.0 gm/dl in case of IDA and 10.3 ± .09 gm/dl in cases of BTT. Mean±SD RBC level was found 4.24 ± 0.76 (10^{12}/L) in case of IDA and 5.35 ± 0.72 (10^{12}/L) in cases of BTT. Mean±SD MCV level was found 72.6 ±6.3 (fl) in case of IDA and 61.8 ± 6.0 (fl) in cases of BTT. Mean±SD MCH was 23.0 ± 2.9 pg in case of IDA and 20.0 ±3.0 in case of BTT patients. Mean±SD MCHC was 30.9 ± 2.1 gm/dl in case of IDA and 31.2 ± 2.0 in case of BTT patients. The mean value of RWD in case of BTT found 15.9 ± 3.1 and 18.0 ±2.5 in case of IDA.

[Table 4] shows the differential values of RDW in favour of IDA >14 and in favour of BTT <14 and RDWI in favour of IDA >220 and in favour of BTT <220.

Table 1: Distribution of the respondents according to Age.

Age (years)	n=110	%	Mean ± SD
1-10	80	72.72	22.0 ± 32.52
11-20	10	10	
21-30	10	10	
31-40	5	4.54	
>40	5	4.54	

Annals of International Medical and Dental Research
E-ISSN: 2395-2822 | P-ISSN: 2395-2814
Vol-8, Issue-3 | May-June 2022
DOI: 10.53339/aimdr.2022.8.3.14
Page no- 107-113 | Section- Research Article (Pathology)

Table 2: Distribution of the respondents according to sex of the patients

Sex	n	%
Male	59	53.6
Female	51	46.4

Table 3: Different values of red blood cell parameters in case of IDA and BTT patients.

Test	Cases	Mean ± SD
Hb (gm/dl)	BTT	10.3 ± .09
	IDA	8.9 ±1.0
RBC (10^{12}/L)	BTT	5.35 ± 0.72
	IDA	4.24 ± 0.76
MCV (fl)	BTT	61.8 ± 6.0
	IDA	72.6 ±6.3
MCH (pg)	BTT	20.0 ±3.0
	IDA	23.0 ± 2.9
MCHC (gm/dl)	BTT	31.2 ± 2.0
	IDA	30.9 ± 2.1
RDW (%)	BTT	15.9 ± 3.1
	IDA	18.0 ±2.5

Table 4: Value of discrimination indices used in evaluation of IDA BTT.

Indices	In favour of IDA	In favour of BTT
RDW (%)	>14	<14
RDWI (MCV X RDW / RBC)	>220	<220

Table 5: Sensitivity, specificity, PPV, NPV and YI of two discrimination indices in diagnosis of BTT and IDA

Indices	Differential value	Cases (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
RDWI (MCV x RDW/ RBC)	<220	BTT	81.0	83.8	81.0	83.0
	>220	IDA	83.8	81.0	81.6	81.0
RDW (%)	<14	BTT	18.0	83.5	48.0	44.10
	>14	IDA	83.5	18.0	44.10	48.0

[Table 5] shows, RDWI had both sensitivity and specificity more than 81% in detection of BTT and IDA. Sensitivity, specificity of RDWI index for detection of BTT was found 81.0%, 83.8%. In case of IDA, sensitivity and specificity was found 83.8% and 81.0% respectively. On the other hand the sensitivity, specificity of RDW for BTT cases were found, 18.0%, and 83.5% respectively and for IDA sensitivity was 83.5% and specificity was 18.8%.

DISCUSSION

IDA and BTT are the most frequent conditions associated with mild microcytic anemia. Microcytic anemias have different prognoses

Annals of International Medical and Dental Research
E-ISSN: 2395-2822 | P-ISSN: 2395-2814
Vol-8, Issue-3 | May-June 2022
DOI: 10.53339/aimdr.2022.8.3.14
Page no- 107-113 | Section- Research Article (Pathology)

and treatments, so differential diagnosis is important in clinical practice. The initial step in diagnosing microcytic anemias is to examine blood smear samples and use cell counters to calculate erythrocyte indices.

The sample size of the recently conducted study was 110. Among them 46 cases were identified as BTT and 64 were IDA.The recent conducted study shows that among 110 respondents 46.4% were female and 53.6% were male. 72.72% respondents were aged between 1-10 years and 4.54% were aged between 31-40 years and also more than 40 years of age. Mean (±SD) age was 22.0 ± 32.52. Another study found a total of 129 patients with microcytic anemia were involved in a retrospective study, 80 with IDA and 49 with BTT.[14] The mean value of RDW, found in IDA and BTT were 18.8±2.5 SD and 15.9±3.1SD respectively. The sensitivity, specificity of RDW in detection of BTT were found, 18.0% and 83.5% and for IDA sensitivity was 83.5% and specificity was 18.0%. RDWI had both sensitivity and specificity more than 80% in detection of BTT and IDA. Sensitivity, specificity of RDWI index for detection of BTT was found 81.0%, 83.8%. In case of IDA, sensitivity and specificity was found 83.8% and 81.0% respectively. Which is reliable discriminator between β thalassaemia trait and iron deficiency anaemia. This results are similar with another study by Demir et al,[7] and Sirdah et al.[13]

The differentiation between IDA and BTT is important because if BTT is misdiagnosed as IDA and treated, it will not normalize in MCV.[8] A better of tests, including calculation of HbA2, peripheral blood film, serum ferritin, iron, TIBC (total iron binding capacity) levels, and transferrin saturation, is required to distinguish between beta thalassaemia trait (BTT) and iron deficiency anemia (IDA).[3] However, these approaches are relatively expensive, time demanding, and specialized. Electronic cell counters can successfully identify microcytosis caused by IDA and BTT using red blood cell volume distribution (RDW) curves.[16]

In this study, RDWI came out as good discriminators between BTT and IDA, had both sensitivity and specificity more than 80% in detection of BTT and IDA. Sensitivity, specificity of RDWI for detection of BTT was found 81.0%, 83.8%. In case of IDA, sensitivity and specificity was found 83.8% and 81.0% respectively. On the other hand the sensitivity, specificity of RDW for BTT cases were found, 18.0%, and 83.5% respectively and for IDA sensitivity was 83.5% and specificity was 18.8%. These results are consistent with the findings of Nesa A et al.[17]

In contrast, a study conducted by Matos et al. reported that RDW was significantly higher in IDA and can be a useful parameter in the discrimination between RDW and IDA. Piriyakhuntorn et al. also conducted an interesting study with a large sample size in Thailand, and concluded that an RDW value of > 21% is a useful in the differentiation between the two.[18,19]

We suggest that geographical area and ethnic differences may influence the molecular spectrum of BTTs and might explain the variations in findings between studies. Based on the high sensitivity, specificity, PPV, and NPV results from both sets of patients, the RDWI is expected to perform well as a

Annals of International Medical and Dental Research
E-ISSN: 2395-2822 | P-ISSN: 2395-2814
Vol-8, Issue-3 | May-June 2022
DOI: 10.53339/aimdr.2022.8.3.14
Page no- 107-113 | Section- Research Article (Pathology)

screening tool for patients with hypochromic microcytic anemia to differentiate case between BTT and IDA.

CONCLUSIONS

From this study it can be concluded that, RDWI appears to be reliable and useful index for initial screening and differationtiation of IDA and BTT.

Recommendation

This study can serve as a pilot to a much larger research that can provide a nationwide picture to ensure better management and adherence.

I am very grateful to many colleagues for their thorough, helpful and usually prompt response to requests for their opinion and advice.

REFERENCES

1. Lazarte SS, Mónaco ME, Jimenez CL, Ledesma Achem ME, Terán MM, Issé BA. Erythrocyte Catalase Activity in More Frequent Microcytic Hypochromic Anemia: Beta-Thalassemia Trait and Iron Deficiency Anemia. Adv Hematol. 2015;2015:343571. doi:10.1155/2015/343571
2. Rahim F, Keikhaei B. Better differential diagnosis of iron deficiencyanemia from beta-thalassemia trait. Turk J Haematol. 2009;26(3):138-45.
3. Lima CS, Reis AR, Grotto HZ, Saad ST, Costa FF. Comparison of red cell distribution width and a red cell discriminant function incorporating volume dispersion for distinguishing iron deficiency from beta thalassemia trait in patients with microcytosis. Sao Paulo Med J. 1996;114(5):1265-9. doi: 10.1590/s1516-31801996000500005.
4. Muñoz M, García-Erce JA, Remacha ÁF. Disorders of iron metabolism. Part II: iron deficiency and iron overload. J Clin Pathol. 2011;64(4):287-96. doi: 10.1136/jcp.2010.086991.
5. Chrobak L. Microcytic and hypochromic anemias. VintrLek. 2001;47:166–174.
6. Ferrara M, Capozzi L, Russo R, Bertocco F, Ferrara D. Reliability of red blood cell indices and formulas to discriminate between beta thalassemia trait and iron deficiency in children. Hematology. 2010;15(2):112-5. doi: 10.1179/102453310X12583347010098.
7. Demir A, Yarali N, Fisgin T, Duru F, Kara A. Most reliable indices in differentiation between thalassemia trait and iron deficiency anemia. Pediatr Int. 2002;44(6):612-6. doi: 10.1046/j.1442-200x.2002.01636.x.
8. Ntaios G, Chatzinikolaou A, Saouli Z, Girtovitis F, Tsapanidou M, Kaiafa G, et al. Discrimination indices as screening tests for beta-thalassemic trait. Ann Hematol. 2007;86(7):487-91. doi: 10.1007/s00277-007-0302-x.
9. Sirdah M, Tarazi I, Al Najjar E, Al Haddad R. Evaluation of the diagnostic reliability of different RBC indices and formulas in the differentiation of the beta-thalassaemia minor from iron deficiency in Palestinian population. Int J Lab Hematol. 2008;30(4):324-330. doi: 10.1111/j.1751-553X.2007.00966.x.
10. Tiwari AK, Chandola I. Comparing prevalence of Iron Deficiency Anemia and Beta Thalassemia Trait in microcytic and non-microcytic blood donors: suggested algorithm for donor screening. Asian J Transfus Sci. 2009;3(2):99-102. doi:10.4103/0973-6247.53883
11. McClure S, Custer E, Bessman JD. Improved detection of early iron deficiency in nonanemic subjects. JAMA. 1985;253(7):1021-3.
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):665-669. doi:10.12669/pjms.333.12098
13. Sirdah M, Tarazi I, Al Najjar E, Al Haddad R. Evaluation of the diagnostic reliability of different RBC indices and formulas in the differentiation of the beta-thalassaemia minor from iron deficiency in Palestinian population. Int J Lab Hematol.

Annals of International Medical and Dental Research
E-ISSN: 2395-2822 | P-ISSN: 2395-2814
Vol-8, Issue-3 | May-June 2022
DOI: 10.53339/aimdr.2022.8.3.14
Page no- 107-113 | Section- Research Article (Pathology)

2008;30(4):324-330. doi: 10.1111/j.1751-553X.2007.00966.x.
14. Janel A, Roszyk L, Rapatel C, Mareynat G, Berger MG, Serre-Sapin AF. Proposal of a score combining red blood cell indices for early differentiation of beta-thalassemia minor from iron deficiency anemia. Hematology. 2011;16(2):123-7. doi: 10.1179/102453311X12940641877849.
15. AlFadhli SM, Al-Awadhi AM, AlKhaldi D. Validity assessment of nine discriminant functions used for the differentiation between iron deficiency anemia and thalassemia minor. J Trop Pediatr. 2007;53(2):93-7. doi: 10.1093/tropej/fml070.
16. Johnson CS, Tegos C, Beutler E. Thalassemia minor: routine erythrocyte measurements and differentiation from iron deficiency. Am J Clin Pathol. 1983;80(1):31-6. doi: 10.1093/ajcp/80.1.31.
17. Nesa A, Tayab MA, Sultana T, Khondker L, Rahman MQ, Karim MA, et al. RDWI is better discriminant than RDW in differentiation of iron deficiency anaemia and beta thalassaemia trait. Bangladesh J Child Health. 2009;33(3):100-3.
18. Matos JF, Borges KB, Fernandes AP, et al. RDW as differential parameter between microcytic anemias in" pure" and concomitant forms. J Bras Patol Med Lab. 2015;51(1):22–27.
19. Piriyakhuntorn P, Tantiworawit A, Rattanathammethee T, Chai-Adisaksopha C, Rattarittamrong E, Norasetthada L. The role of red cell distribution width in the differential diagnosis of iron deficiency anemia and non-transfusiondependent thalassemia patients. Hematol Rep. 2018;10(3):7605. doi:10.4081/hr.2018.7605

Source of Support: Nil, Conflict of Interest: None declared