Original Article

Differentiating Iron Deficiency Anemia from Beta-Thalassemia Trait Using Red Cell Indices: A Cross-Sectional Study

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Abstract

Background: *Anemia, particularly iron deficiency anemia (IDA) and beta-thalassemia trait (βTT), is a prevalent health issue worldwide, especially in resource-limited settings like Bangladesh. Accurate differentiation between these two conditions is vital for effective management, as they have distinct pathophysiologies and treatment protocols.*

Objectives: *This study is aimed to evaluate the reliability of red cell indices derived from automated cell counters to distinguish between IDA and βTT, thereby reducing the need for more invasive and expensive diagnostic methods.*

Materials and Methods: *A total of 150 patients with microcytic hypochromic anemia were included in a cross-sectional analytical study conducted at the Department of Hematology, Dhaka Medical College Hospital. Using automated parameters, eight discrimination indices were calculated. Confirmatory diagnoses were made through hemoglobin electrophoresis and serum iron profiles. Sensitivity, specificity, and predictive values for each index were computed.*

Results: *The study identified 60% of the patients as βTT and 40% as IDA. The best-performing indices for differentiation were the Green and King index (94% correct identification), followed closely by the Red Cell Distribution Width Index (RDWI, 93.33%). RBC count and Mentzer index showed similar performance (87.33%). Both RBC count and RDWI demonstrated 100% sensitivity in detecting IDA. Sensitivity, specificity, and predictive values varied significantly among indices, with the Green and King index exhibiting the highest overall accuracy.*

Conclusion: *This study concludes that red cell indices are effective tools for differentiating between IDA and βTT in resource-constrained settings, facilitating timely and appropriate management. Further studies with larger sample sizes are recommended to validate these findings and enhance diagnostic protocols.*

Key words: *Iron Deficiency Anemia (IDA), Beta Thalassemia Trait (βTT), Red Cell Indices, Green and King (G&K), Red Cell Distribution Width Index (RDWI)*

Date of received: *12.04.2025*

Date of acceptance: *30.06.2025*

DOI: https://doi.org/10.3329/kyamcj.v16i02.80833

KYAMC Journal. 2025; 16(02): 53-58.

Introduction

Anemia is the most common disorder of blood. Iron deficiency and thalassemia trait are most common conditions causing microcytic hypochromic anemia. Among them, iron deficiency anemia (IDA) causes almost half of all anemic cases worldwide. Moderate degree of iron deficiency affected approximately 610 million people or 8.8% of the total population and mild degree affects another 375 million worldwide.[1] In Bangladesh, it ranges from 49 to 81%.[2]

Thalassemia is another cause of microcytosis. Though reliable data are still lacking, about 6-7% of total anemic population have hemoglobin disorder.[1] It is estimated that 1.5% of the world population is beta-thalassemia carrier. About 6000 new carriers are born every year and 50% of thalassemia minor is in South East Asia.[2] WHO estimates that 3% are carrier of β-thalassemia and 4% of Hb-E in Bangladesh.[3]

In the last four decades, many formulas have been proposed to discriminate between IDA and thalassemia.[4] Peripheral blood smear, iron profile, Hb electrophoresis, bone marrow stain, zinc protoporphyrin, transferrin receptor are the best methods to differentiate, but all of them are both expensive and sometimes invasive.[4-6] Apart from these methods some authors try to

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introduce cell count base formula such as RBC count, Mentzer formula, England and Fraser (E&F), Green and King (G&K), Shine and Lal (S&L), Red Cell Distribution Width Index (RDWI).

RDWI represent the coefficient of variation of the RBC volume distribution and is considered an index of heterogeneity, the equivalent of anisocytosis. High RDWI goes in favor of IDA and normal RDWI in favor of thalassemia trait.6 RDWI shows promising value to differentiate between IDA and β-thalassemia trait.[6,7] RDWI is measured by simple calculation (MCV × RDW ÷ RBC). RDWI <220 indicates thalassemia trait and >220 is in favor of IDA. In the developing countries like Bangladesh, where majority of people are below poverty line, the ideal methods are expensive and time consuming. For this reason, cell counter based formulas may play a significant role to differentiate IDA from thalassemia trait. Considering the facts, the study was designed to find out the sensitivity and specificity of RBC indices based formula from cell counter analyzer, which in turn would be expected to help minimize the cost of investigations and expenditures of patients.

Red Blood Cell Indices

Electronic automated cell counter analyzer has been used to determine red cell indices. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) were first introduced by Wintrobe in 1929 to define the size (MCV) and hemoglobin content (MCH, MCHC) of red cells. Termed red cell indices, these values can be calculated if the values of hemoglobin, hematocrit and red blood cell count are known. Variation in the size of red cells can be quantified and expressed as red cell distribution width (RDW) or as red cell morphology index.

Mean corpuscular volume (MCV)

The average volume of red cell is expressed in femtoliters (fl). It is a key diagnostic indicator even though the pattern of mean corpuscular hemoglobin (MCH) is usually similar to that of MCV. Virtually all automated hematological analyzer now provide a measurement of MCV that is both precise and accurate. In most adult populations it ranges from 80-100fl. Defective hemoglobin synthesis results in small cells (low MCV) with or without anisocytosis, as the continued cell division leads to microcytosis. So, thalassemic individuals have a reduced MCV and in heterozygous β-thalassemia trait (βTT), the cells are uniformly small. Studies have suggested that the MCV may predict the mutation and thus severity[8] and that a value of 72fl is maximally sensitive and specific for a presumptive diagnosis of β-thalassemia.[9]

Mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC)

MCH quantifies the amount of hemoglobin per red blood cell. The normal values for MCH for MCH are 29 ± 2 picogram (pg) per cell. MCHC indicates the amount of hemoglobin per unit volume. In contrast to MCH, MCHC correlates the hemoglobin content with the volume of the cell. It is expressed as g/dl of red cell or as a percentage value. The normal values for MCHC are 34 ± 2 g/dl.10

Red cell distribution width (RDW)

The RDW is a measure of the degree of anisocytosis. In heterozygous β-thalassemia the cells are uniformly small (low MCV; RDW tends to be normal), whereas in iron deficiency increase RDW may be the first laboratory abnormality, even before anemia and microcytosis are seen.[10-14]

Materials and Methods

Sample

This was a cross-sectional analytical study carried out among 150 patients (age 1 to 60 years of both sex) presented in the Department of Hematology, Dhaka Medical College Hospital during January 2013 to December 2013.

Sampling strategy

Participants were selected through a purposive sampling procedure. Patients of age 1 to 60 years of both sex with the evidence of microcytic hypochromic anemia in cell counter analyzer were included. The patients diagnosed of thalassemia major, concomitant of other hematological diseases were excluded. Even patients having both IDA and βTT at the same time were excluded.

Covariates

We considered the following demographic and lifestyle variables: age (1-10, 11-20, 21-30, 31-40, 41-50 and 51-60), IDA and βTT.

Statistical analysis

All the data were checked and edited after collection. Then data were entered in SPSS 20 for Windows 7. Frequency distribution and normal distribution of continuous variables were calculated and expressed as Mean ± SD. Mean difference between IDA and βTT groups of patients was determined by chi-square test.

Results

The study included a total number of 150 patients of microcytic hypochromic anemia who were having either iron deficiency anemia or beta thalassemia trait. Final diagnosis was confirmed by serum iron profile and hemoglobin electrophoresis. Total 90 cases of βTT (60%) and 60 cases of IDA (40%) were finally diagnosed using above methods.

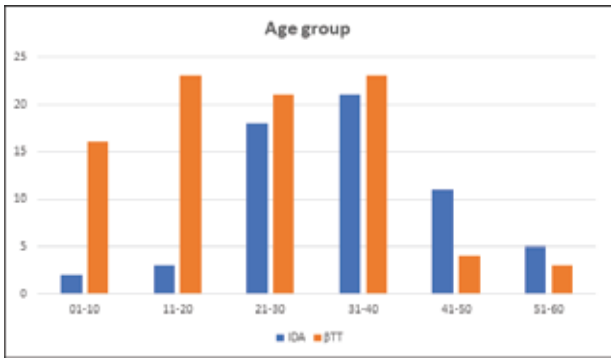

Figure 1: Age group distribution of the subjects (n=150)

Table I: Hematological values of the group (n=150)

Variables	IDA n=60 (Mean ± SD)	βTT n=90 (Mean ± SD)	P value
Hb (g/dl)	8.76 ± 1.68	11.24 ± 1.10	<0.001
RBC (mill/cumm)	4.15 ± 0.59	5.52 ± 0.52	< 0.001
MCV	68.67 ± 4.08	68.30 ± 56.24	0.96
MCH	20.70 ± 2.44	20.47 ± 1.43	0.47
MCHC	29.87 ± 2.57	32.59 ± 1.16	< 0.001
RDW	19.06 ± 3.41	17.00 ± 1.92	< 0.001
Serum Iron	27.87 ± 16.04	81.76 ± 19.16	< 0.001
Serum ferritin	10.49 ± 4.40	79.35 ± 36.38	< 0.001
TIBC	400.73 ± 138.87	322.76 ± 45.97	< 0.001
Hb electrophoresis			
Hb A	97.35 ± 0.63	103.98 ± 89.36	0.56
Hb A2	2.53 ± 0.56	5.42 ± 1.05	<0.001

p value determined by t-test. Hb = hemoglobin, RBC = Red blood cell, MCV = Mean corpuscular volume, MCH = Mean corpuscular hemoglobin, MCHC = Mean Corpuscular Hemoglobin Concentration, RDW = Red Cell Distribution Width, TIBC = Total Iron Binding Capacity

Table II: The differential values of each discrimination index, along with the number of correctly identified patients in each group (IDA and βTT) as well as in the total study population.

Differential Values	IDA (n=60)	βTT (n=90)	Total number of correctly diagnosed patients (n =150) (IDA+βTT)	Percentage of correctly identified patients (%)
RBC				
IDA <5	60	19	131	87.33
βTT >5	0	71		
RDWI				
IDA > 220	60	10	140	93.33
βTT <220	0	80		
G& K				
IDA > 72	54	3	141	94.00
βTT < 72	6	87		
MI				
IDA >13	56	15	131	87.33
βTT <13	4	75		
S I				
IDA >13	57	32	115	76.67
βTT <13	3	58		
S& L				
IDA >1530	0	1	89	59.33
βTT < 1530	60	89		

IDA = Iron Deficiency Anemia, βTT = Beta thalassemia trait, RBC = Red blodd cell, RDWI = Red Cell Distribution Width Index, G&K = Green and King index, MI = Mentzer index, SI = Srivastava index, S&L = Shine and Lal index.

Table III: Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and Youden's index of each discrimination index in prediction of IDA and βTT groups.

Group	Category	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Youden's Index (%)
RBC	IDA	100	79	76	100	76
	βTT	79	100	100	76	
RDW	IDA	100	89	86	100	86
	βTT	89	100	100	86	
G&K	IDA	90	97	95	94	87
	βTT	97	90	94	95	
MI	IDA	93	83	79	95	77
	βTT	83	93	95	79	
S I	IDA	95	64	64	95	59
	βTT	64	95	95	64	
S&L	IDA	0	99	0	60	- 1
	βTT	99	0	60	0	

Age group distribution of subjects according to confirmed diagnosis shows that βTT frequency was higher among younger groups and IDA had a higher frequency in older age groups. Age 21-30 and 31-40 showed nearly similar distribution of IDA and βTT patients. This differences in distribution was significant (p value <0.001 as derived by Chi-square test) as shown in Figure 1.

Among 150 subjects there were 53.3% (80) male patients and 46.7% (70) female patients. Among 60 cases of IDA, 36.7% were male and 63.3% female. So, IDA was found more in female patients and βTT was found more in male patients.

In Table I, mean hemoglobin, RBC count, MCHC, serum iron, ferritin, hemoglobin A2 were significantly lower in IDA than β TT. Mean red cell distribution width (RDW) and total iron binding capacity saturation was significantly higher in IDA than that of βTT. Distribution of MCV and hemoglobin A was similar across both groups.

The differential values of each discrimination index are given in Table II. The differential values for each discrimination index were applied as defined in the original published reports. It also shows the total number of correctly diagnosed patients along with percentage among total patients. The most accurate index in terms of percentage of correctly identified patients was Green & King index (G&K, 94%). It is followed in decreasing order by Red Cell Distribution Width Index (RDWI, 93.33%), Red blood cell count (RBC, 87.33%), Mentzer index (MI, 87.33%), Srivastava index (SI, 76.67%) and Shine and Lal index (S&L, 59.33%). Among all, the S&L index was the most inaccurate.

Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of each index are presented in Table III. RBC and RDWI showed 100% sensitivity in the detection of IDA and 79% and 89% sensitivity respectively in the diagnosis of βTT. G&K index showed respectively 90% and 97% sensitivity in the detection of IDA and βTT. Mentzer index showed 93% and 83% sensitivity in diagnosing IDA and βTT, respectively. Srivastava index showed 95% sensitivity for IDA and 64% sensitivity for βTT. S&L was the least sensitive index of all. Youden's index revealed G&K index to be most accurate (87%), followed by RDWI (86%) and RBC (76%).

Discussion

Both IDA and βTT cause microcytic hypochromic anemia. So, it would be beneficial in the context of low and middle income country like Bangladesh, if a formula could be made which can differentiate between the two diseases only by looking at the blood count results. Various formulas based on Hb, RBC count, MCV and RDW have been devised in an attempt to separate IDA from βTT without going for hemoglobin electrophoresis and serum iron profile in all patients.[15-17]

In this study, among 150 patients 60% patients had βTT and 40% had IDA which is nearly same as the findings reported in another study by Aysin Demir and his colleagues in Turkey.13 Their study only included children and they found 41% IDA patients and 59% βTT patients. Whereas a study conducted to determine frequency of IDA and βTT among patients of microcytic anemia in India found respectively 52% and 36% cases of IDA and βTT in their subjects.[18]

Mean age of all the subjects was 27.54±13.01 years. Mean age

of IDA subjects were higher (33.81±12.05) than that of βTT (23.36±11.95). βTT is a genetically inherited autosomal recessive disorder and usually does not cause symptomatic anemia. As a result, most of the cases are found to have thalassemia as part of screening process of the family members or incidentally during other illness. Whereas, IDA may occur as a result of deficient in iron rich diet or continuous blood loss.

Mean hemoglobin, RBC count, MCHC, serum iron, serum ferritin, hemoglobin A2 were significantly lower in IDA than that of βTT. Mean RBC count and hemoglobin was also found significantly lower in IDA patients then β-thalassemia minor patients in the study conducted by Miri-Moghaddam et al[19] but their study found significantly lower MCV in IDA too. This was not the case in this study, where mean MCV was nearly same in both groups. Mean serum iron was also found lower in IDA patients by Demir et al.[13]

Mean red cell distribution width (RDW) along with iron binding capacity and saturation was significantly higher in IDA patients than that of βTT patients. Mean RDW was also found higher in IDA patients than βTT by Flynn et al in their study.[20]

Comparison of sensitivity, specificity, PPV, NPV and Youden's index of different formulas shows that the best indices for differentiating IDA from βTT from blood count variables is G&K, followed by RBC count, RDWI and Mentzer index. Nearly similar observation was reported by Demir et al.[13] They also reported RBC and RDWI as the best indices. They found sensitivity for RBC and RDWI in detecting IDA of 96% and 80% respectively. In contrast the present study found a very high accuracy for both RBC count and RDWI in detecting IDA (100% sensitivity for both). On the other hand, Demir et al[13] reported respectively 86% and 100% specificity for differentiating IDA from βTT by RBC and RDWI. Whereas, in this study a specificity of 79% and 89% respectively was found for those tests. The Youden's index was found 76% and 86% for RBC and RDWI respectively in this study. In contrast, Demir and colleagues found the values to be 82% and 80% respectively.

Conclusion

Different formulas are used now-a-days but to differentiate IDA from βTT, full blood counts from auto analyzers are one of the usable tools when the tests for diagnostic confirmation are not available or cannot be afforded by the patient. Detection of βTT trait is necessary for genetic counseling and IDA is necessary to detect a potentially treatable cause. This study found G&K index, RBC count, RDWI and Mentzer index to be more useful discriminating indices. On the other hand, Srivastava Index was found less useful and Shine and Lal index wasn't found to be useful at all. Moreover, as the study is cross-sectional in nature, so further cohort population-based study is necessary to conclude and validate the findings.

Acknowledgement

We would like to thank all the faculty members of Department of Hematology of Dhaka Medical College Hospital and Department of Transfusion Medicine of KYAMCH for their support and co-operation.

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