ORIGINAL RESEARCH

Reliability Of Different RBC Indices To Differentiate Between Beta Thalassemia Trait And Iron Deficiency Anemia In Children

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ABSTRACT

Aim: To evaluate the reliability of different RBC indices to differentiate between Beta thalassemia trait and iron deficiency anemia in children. **Materials and Methods:** The present prospectivestudy was conducted in Department of Paediatrics, Mahatma Gandhi Medical College & Hospital, Jaipur from March 2021 to July 2022 among 105 patients admitted in Department of Paediatrics for anemia. Erythrocyte microcytosis and hypochromia was assessed using automated blood cell counter and by peripheral smear. All cases with normal MCV and MCH will not be tested further. The cases with reduced level of serum ferritin were labelled as IDA while those with normal or increased serum ferritin level were subjected to HPLC examination. 10 indices were calculated among both groups using CBC parameters. Sensitivity and specificity, PPV, NPV, DA &Youden's index for these indices were calculated.**Results**: 91 (86.67%) subjects had Iron Deficiency Anemia and 14 (13.33%) patients were of Thalassemia. RDW, Mentzer index and Kerman 1 index gave the correct diagnosis in 97.14%, 95.24% and 95.23% of the patients respectively. Rest all the indexes gave accuracy of >80% except SHINE AND LAL INDEX which gave accuracy of only 19.05%. None of the indices studied showed 100% sensitivity or specificity. However, SLIMCVXMCVXMCH/100 index demonstrated highest sensitivity (98.3%) to detect β thalassemia trait but low specificity (8.4%). RDW index showed second highest sensitivity (84.9%) followed by MENTZERS (MCV/RBC). **Conclusion**: Result of present research leads to conclusion that the percentage of correctly diagnosed patients was highest with RDW followed by Mentzer index, RDW and Kerman 1 index. The results of the current study highlight the fact that red cell indices are now reasonably sensitive, specific, reproducible, and exact and can be relied upon for usage in peripheral health clinics without access to more expensive tests like electrophoresis and HPLC. Additionally, it can reduce the costs associated with β-Thalassemia mass screening.

Keywords: Anemia, IDA, Beta thalassemia, RDW

INTRODUCTION

Morphologically, microcytic anemias are among the most common type of anemias encountered by physicians and the two most common causes of microcytic anemia are IDA and Thalassemia trait (TT). IDA is characterized by pallor, fatigue and weakness. In severe IDA patient may present with koilonychia, alopecia, atrophic changes in the tongue & gastric mucosa. [1]

β-Thalassemia is an important disorder that has attracted the attention of medical research towards the multifaceted disease. [2-4] Most of the studies in the past have focused on β-thalassemia which is one of the most common single gene disorder with >4,00,000 new borns affected per year worldwide. [5] It is an autosomal hematological disorder resulting from genetically deficient synthesis of β-globin chain of Hb which leads to an excessive accumulation of α- globin chains. The extra α-globin chain precipitates and forms inclusion bodies in RBCs, which may be apparent in mildest β-Thalassemia Trait (BTT). The BTT patients are usually asymptomatic. They may

present with mild anemia, headache or leg cramps. Peripheral blood smear shows same picture as IDA. [6]

It is important to differentiate between the IDA and BTT because each has entirely different etiology, pathogenesis, treatment and prognosis. Thus an unknown number of misdiagnosed thalassemia may be unnecessarily treated with iron. The real danger of misdiagnosed or non-diagnosis in carriers of BTT getting married, is a potential homozygous off spring.

In the past, various indices have been proposed todiscriminate IDA from BTT according to geographicalvariations. These discrimination indices proposed bydifferent workers are derived from different haematological parameters. [7] In past several attempts have been made to develop a discrimination index which is suitable for discrimination between IDA and BTT. Some of these indices are – Shine and Lal, [8]Mentzer, [9] England and Fraser, [10] Srivastava, [11] Green and King, [12] Ricer et al., [13] and Red Cell Distribution Width (RDW), [14] among many.

High performance liquid chromatography (HPLC) forms an important gold standard tool for screening and detection of various hemoglobinopathies with rapid, reproducible and precise results. [15] However, this test is not available at many peripheral centers. Electronic cell counters have been used to determine red cell indices as a first indicator of b-TT. Since 1970, a number of complete blood count indices have been proposed as simple and inexpensive tools to determine whether a blood sample is more suggestive of b-TT or IDA. [16] An ideal discrimination index has high sensitivity and specificity; that is, it can detect the maximum number of patients with b-TT (high sensitivity) while eliminating patients with IDA (high specificity). Youden's index was suggested by W.J. Youden and is a way of summarising the performance of a diagnostic test and is calculated by substracting 100 from the sum of sensitivity and specificity. [17,18]

Both β-TT and IDA have an entirely different cause, prognosis, and treatment. Hence, distinguishing them has important clinical implications. However, no single marker or any combination of tests has been found to be optimal for this discrimination.Hence the present study was undertaken to compare the utility of various hematological discrimination indices in differentiating BTT from IDA by calculating their sensitivity, specificity, PPV, NPV, Diagnostic Accuracy and Younden's index.

MATERIALS AND METHODS

The present prospectivestudy was conducted in Department of Paediatrics, Mahatma Gandhi Medical College & Hospital, Jaipur from March 2021 to July 2022 among 105 patients admitted in Department of Paediatrics for anemia.The ethical clearance for study was taken by the Institutional Review Board for Ethical Clearance of Mahatma Gandhi Medical College & Hospital, Jaipur. All consenting patient's attendants were asked to sign a written informed consent form (in the language best understood by them).

INCLUSION CRITERIA

1. Age >1 year and <18 years of either gender.
2. Hb level <10gm/dl.
3. MCV <80fl and MCH <25pg.

EXCLUSION CRITERIA

1. Diagnosed cases of any other hemoglobinopathies.
2. Acute bleeding episodes in previous months.
3. Recent blood transfusion

METHODOLOGY

- A record of clinical profile was made using predesigned proforma after obtaining consent.
- Erythrocyte microcytosis and hypochromia was assessed using automated blood cell counter and by peripheral smear. All cases with normal MCV and MCH will not be tested further.
- All samples which were microcytic (MCV <80fl) and hypochromic (MCH<27pg) was tested for serum ferritin level (as per age) by ELIZA method.
- The cases with reduced level of serum ferritin were labelled as IDA while those with normal or increased serum ferritin level were subjected to HPLC examination.
- 10 indices were calculated among both groups using CBC parameters.
- Sensitivity and specificity, PPV, NPV,DA & Youden's index for these indices were calculated.

INDICES	FORMULA	B-TT	IDA
RDW	B-TT= <13	<13	>13
MENTZERS	(MCV/RBC)	<13	>13
EFI	MCV-(5*Hb)-RBC-3.4	<0	>0
SLI	MCV*MCV*MCH/100	<1530	>1530
GKI	MCV*MCV*RDW/Hb*100	<65	>65
SRIVASTAVA	MCV/RBC	<3.8	>3.8
RDW INDEX	MCV*RDW/RBC	<220	>220
SIRDAH	MCV-RBC-(3Hb)	<27	>27
EHSANI	MCV-(10*RBC)	<15	>15
	MCV*MCH/RBC	<300	>300

Sensitivity = [TP/TP+FN]*100
Specificity = [TN/TN+FP]*100
PPV = [TP/TP+FP]*100
NPV = [TN/TN+FN]*100
DA =[TP+TN/TP+FP+TN+FN]*100
Youden's Index = (sensitivity +specificity)-100

STATISTICAL ANALYSIS

Data so collected was tabulated in an excel sheet, under the guidance of statistician. The means and standard deviations of the measurements per group were used for statistical analysis (SPSS 22.00 for windows; SPSS inc, Chicago, USA). For each assessment point, data were statistically analyzed using one way ANOVA. Difference between two groups was determined using chi square test and the level of significance was set at $p < 0.05$.

RESULTS

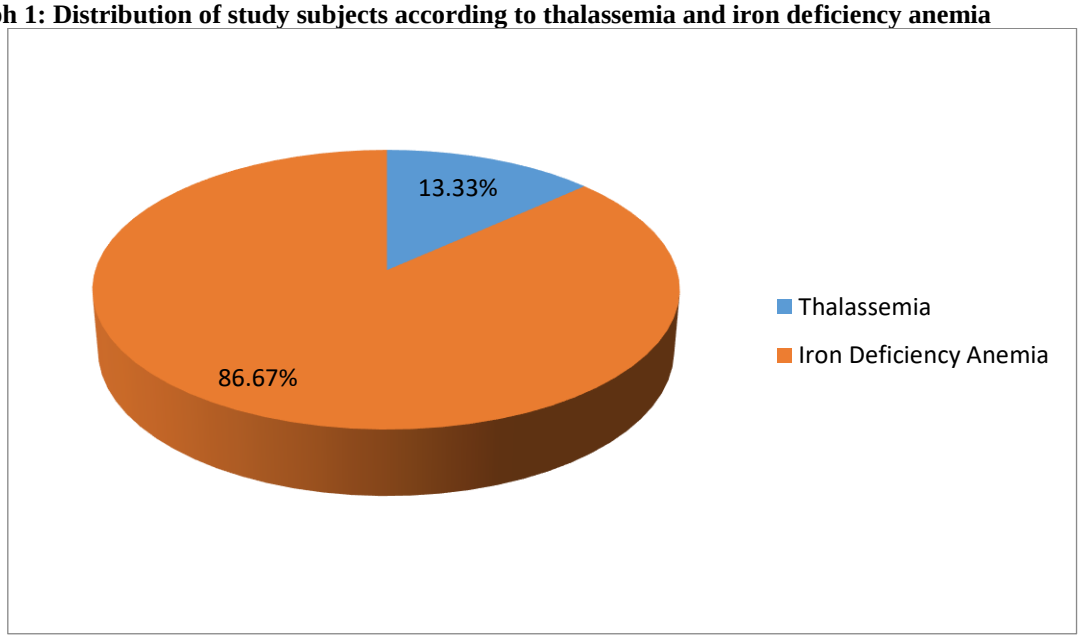
Of the 105 subjects included in the study, 54 (51.43%) were females and 51 (48.57%) were males.Mean age of the study subjects was 8.29±3.65 years. (Table 1)

Table 1: Gender distribution and mean age among the study subjects

Gender	N	%
Male	51	48.57
Female	54	51.43
Total	105	100
Variables	**Age (in years)**	
Mean	8.29	
SD	3.65	
Median	8	

Among 105 subjects, 91 (86.67%) subjects had Iron Deficiency Anemia and 14 (13.33%) patients were of Thalassemia. (graph 1)

Graph 1: Distribution of study subjects according to thalassemia and iron deficiency anemia



Mean Hb level in subjects of Thalassemia was 9.62±0.29 (median value 9.7) and in patients of Iron Deficiency Anemia 7.82±1.37 (median value 8). There was a statistically significant difference present in mean Hb level in subjects of Thalassemia when compared with patients of Iron Deficiency Anemia ($p<0.01$). Mean RBC level in subjects of Thalassemia was 5.58±1.23 (median value 5.02) and in patients of Iron Deficiency Anemia 3.91±0.43 (median value 3.5). There was a statistically significantdifference present in meanRBC level in subjects of Thalassemia when compared with patients of Iron Deficiency Anemia ($p<0.01$). (Table 2)

Table 2: Comparison of Hb and RBC among the study subjects

Parameters		Hb	RBC
Thalassemia	Mean	9.62	5.58
	SD	0.29	1.23
	Median	9.7	5.02
Iron Deficiency Anemia	Mean	7.82	3.91
	SD	1.37	0.43

	Median	8	3.5
p value		**<0.01***	**<0.01***

***:** statistically significant

Mean MCV level in subjects of Thalassemia was 66.88±5.40 (median value 67.2) and in patients of Iron Deficiency Anemia 73.62±3.18 (median value 74.2). There was a statistically significantdifference present in meanMCV level in subjects of Thalassemia when compared with patients of Iron Deficiency Anemia (p<0.01). Mean MCH level in subjects of Thalassemia was 21.06±1.93 (median value 21.1) and in patients of Iron Deficiency Anemia 24.58±2.53 (median value 23). There was a statistically significantdifference present in meanMCHlevel in subjects of Thalassemia when compared with patients of Iron Deficiency Anemia (p<0.01). Mean RDW level in subjects of Thalassemia was 17.4±3.12 (median value 16.1) and in patients of Iron Deficiency Anemia 16.9±1.41 (median value 16). There was no significantdifference present in meanRDW level in subjects of Thalassemia when compared with patients of Iron Deficiency Anemia. (Table 3)

Table 3: Comparison of MCV, MCH and RDW among the study subjects

Parameters		**MCV**	**MCH**	**RDW**
Thalassemia	Mean	66.88	21.06	17.4
	SD	5.40	1.93	3.12
	Median	67.2	21.1	16.1
Iron Deficiency Anemia	Mean	73.62	24.58	16.90
	SD	3.18	2.53	1.41
	Median	74.2	23	16
p value		**<0.01***	**<0.01***	0.46

***: statistically significant**

RDW, Mentzer index and Kerman 1 index gave the correct diagnosis in 97.14%, 95.24% and 95.23% of the patients respectively. Rest all the indexes gave accuracy of >80% except SHINE AND LAL INDEX which gave accuracy of only 19.05%. (Table 4)

Table 4: Correctly diagnosed cases by different RBC indices

INDEX	**TT**	**IDA**	**Correctly Diagnosed**	
			N	**%**
MENTZER				
β-T<13	10	1		95.24
IDA>13	4	90	100	
RDW				
β-T<220	12	1		97.14
IDA>220	2	90	102	
SHINE AND LAL INDEX				
β-T<1530	13	86		19.05
IDA >1530	1	7	20	
SRIVASTAVA INDEX				
β-T<3.8	5	6		85.71
IDA>3.8	9	85	90	
GREEN AND KING INDEX				
β-T<65	11	13		86.67
IDA>65	3	78	91	
SIRDAH INDEX				
β-T<27	5	3		88.57
IDA>27	9	88	93	
EHSANI				
β-T<15	5	2		89.52
IDA>15	9	89	94	
ENGLAND FRASER INDEX				
β-T<0	4	2		88.57
IDA>0	10	89	93	
RDW				
β-T<13	3	11		79.05
IDA>13	11	80	83	
KERMAN1				

β-T<300 IDA>300	10 4	1 90	 100	95.23

None of the indices studied showed 100% sensitivity or specificity. However, SLIMCVXMCVXMCH/100 index demonstrated highest sensitivity (98.3%) to detect β thalassemia trait but low specificity (8.4%). RDW index showed second highest sensitivity (84.9%) followed by MENTZERS (MCV/RBC). Mentzer index, England and Fraser Index, RDW index and Ehsani index each, showed high specificity (99.6% each). Moreover, RDW index has highest positive predictive value (90.8%), while Green and King Index have highest negative predictive value (98.8%). Youden's index showed following ranking with respect to the indices' ability to distinguish between β thalassemia trait and iron deficiency anemia- highest for Kerman 1 index (65.3%) ≥ RDW index (63%) ≥ Green and King index (56.2%) ≥ Mentzer index (42.4%) ≥Ehsani index (36.1%) ≥ Sirdah index (35.9%) ≥Srivastava index (35.4%) > England and Fraser index (24.9%) ≥ RDW (0.2%). (Table 5)

Table 5: Reliability of different RBC indices to differentiate between Thalassemia and Iron Deficiency Anemia

Index	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	LR+	LR-	Accuracy	Youden's Index
RDW	84.9	92.2	5.1	94.9	1.0	0.9	98	0.2
MENTZERS (MCV/RBC)	81.5	99.6	87.0	97.0	127.9	0.5	95	42.4
EFIMCV-(5XHb)-RBC-3.4	25.3	99.6	79.9	96.1	75.7	0.1	95	24.9
SLIMCVXMCVXMCH /100	98.3	8.4	5.3	97.9	1.0	0.1	12	6.7
GKIMCVXMCVXRDW/HbX100	60.2	96.0	44.6	98.8	15.2	0.3	94	56.2
SRIVASTAVAMCH/RBC	36.4	98.9	65.6	96.7	36.2	0.6	99	35.4
RDW Index MCVXRDW/RBC	63.4	99.6	90.8	98.0	189.4	0.3	97	63.0
SIRDAHMCV-RBC-(3Hb)	36.4	99.5	82.1	96.7	87.1	0.6	96	35.9
EHSANIMCV-(10XRBC)	36.4	99.6	85.1	96.7	108.9	0.6	96	36.0
KERMAN1 MCVXMCH/RBC	66.6	98.8	74.9	98.2	56.8	0.3	97	65.3

DISCUSSION

Among the spectrum of β-thalassemia syndrome forms, β-Thalassemia trait (BTT) is the most common form encountered in India and is responsible for causing anemia other than IDA. However, a traditional approach followed by most general practitioners and blood bank physicians is a trial of iron treatment whenever anemia and/or microcytosis are encountered. [19] However, this approach leads to unnecessary iron therapy/iron overload leading to oxidative stresses which leads to deterioration of patient instead of providing a cure. One of the reasons for adaptation of this approach is that burden of BTT is not exactly reported widely and at the same time the diagnosis of β-thalassemia is a cumbersome task which has both time as well as financial implications. [20] In such a scenario, a hematologist is concerned about reducing the burden of cost and time in order to diagnose β-thalassemia. To avoid much more expensive, time-consuming, and complicated procedures for discrimination between these disorders, researchers attempt to use either RBC indices such as MCV, MCH, and RDW, or formulas derived from these indices. This process helps to select appropriate individuals for more detailed examination.

In the present study, of the 105 subjects included in the study, 54 (51.43%) were females and 51 (48.57%) were males. There was a slight female predominance among study subjects. Mean age of the study subjects was 8.29±3.65 years. Similar age and gender distribution was reported by **Soliman AR et al., (2014)** [21] and **Vehapoglu A et al., (2014)** [16].

In present study, among 105 subjects, 91 (86.67%) subjects had Iron Deficiency Anemia and 14 (13.33%) patients were of Thalassemia. In study done by **Ahmad S et al., (2021)** [125] out of 407 subjects selected for the study, 375 (92.1%) had IDA, 15 (3.7%) were β-Thalassemia Trait (BTT), 5 (1.2%) were positive for thalassemia major.

In this research, the amount of microcytosis and hypochromia was more in β-TT than IDA. Mean RDW level in subjects of Thalassemia was 17.4±3.12 (median value 16.1) and in patients of Iron Deficiency Anemia 16.9±1.41 (median value 16). The RDW was increased in both groups. There was no significantdifference present in meanRDW level in

subjects of Thalassemia when compared with patients of Iron Deficiency Anemia. In a study by **Vehapoglu A et al., (2014)** [16],similar findings were revealed. In a study done by **Ahmad S et al., (2021)** [22]; mean MCV, RDW, % RDW and S. TIBC levels of BTT patients was significantly lower as compared to IDA patients while mean hemoglobin level, S. Iron, S. Ferritin levels of BTT patients was significantly higher as compared to that of IDA patients ($p<0.05$). For all the other parameters, statistically, no significant difference between two groups was observed ($p>0.05$)

In study done by **Bhushan R et al., (2018)** [23], RDW was increased in both groups: 17.2 + 3.16 in the IDA group and 16.94 + 1.57 in patients with β-TT ($P > 0.05$). RBC count reduced in IDA (3.7 + 0.27) while it was mildly elevated in β-TT (5.6 + 1.08) and this difference was statistically significant ($P <0.05$)

None of the indices studied showed 100% sensitivity or specificity. However, SLIMCVXMCVXMCH/100 index demonstrated highest sensitivity (98.3%) to detect β thalassemia trait but low specificity (8.4%). RDW index showed second highest sensitivity (84.9%) followed by MENTZERS (MCV/RBC). Mentzer index, England and Fraser Index, RDW index and Ehsani index each, showed high specificity (99.6% each). Moreover, RDW index has highest positive predictive value (90.8%), while Green and King Index have highest negative predictive value (98.8%). Youden's index showed following ranking with respect to the indices' ability to distinguish between β thalassemia trait and iron deficiency anemia- highest for Kerman 1 index (65.3%) ≥ RDW index (63%) ≥ Green and King index (56.2%) ≥ Mentzer index (42.4%) ≥Ehsani index (36.1%) ≥ Sirdah index (35.9%) ≥Srivastava index (35.4%) > England and Fraser index (24.9%) ≥ RDW (0.2%).

Shine and Lal index demonstrated highest sensitivity in the present study, this was similar to the study done by **Bhushan R et al., (2018)** [23] (98.4%), Boardbar E et al., (2015) [24] (87.6%) and **Batebi A et al., (2012)** [25] (83.1%). **Bain BJ (1988)** [26] reported that Shine and Lal index successfully identified 57 of 58 index pregnancies in patients with beta thalassaemia trait. In study done by **Ahmad S et al., (2021)** [22] evaluation of Shine and Lal Index revealed the sensitivity, specificity, PPV, NPV, and accuracy to be 80%, 23.7%, 4%, 96.7% and 25.9% respectively.

Mentzer index in present study showed highest specificity, which was similar to findings of **Bhushan R et al., (2018)** [23] (99.66%), **Batebi A et al., (2012)** [25] (85.4%). In study done by **Ahmad S et al., (2021)** [22] evaluation of Mentzer Index revealed the sensitivity, specificity, PPV, NPV, and accuracy to be 46.7%, 97.6%, 43.8%, 97.8% & 95.6% respectively.

Present study showed sensitivity and specificity of RDW as 7.9% and 92.2% respectively. **Garg S et al., (2016)** [18] showed specificity of this index as 94.8%. In present study Youden's index was highest for Kerman 1 index (65.3%) and RDW index (63%). But **Demir A et al., (2002)** [27] showed that Youden's indices of RBC count and RDW were the highest, with values of 82%and 80% respectively. **Vehapoglu A et al., (2014)** [16] showed that Mentzer index had the highest Youden's index for correctly distinguishing β-Thalassemia and IDA (81%). In study done by **Ahmad S et al., (2021)** [22] Youden's J statistic was observed to be having a performance level of 44.3%.

RDW, Mentzer index and Kerman 1 index gave the correct diagnosis in 97.14%, 95.24% and 95.23% of the patients respectively. Rest all the indexes gave accuracy of >80% except SHINE AND LAL INDEX which gave accuracy of only 19.05%. If a patient with microcytic anemia has correct measures RDWI, show β TT or IDA it is very likely that the diagnosis is correct. However, in a small number of patients it would still be necessary to study body iron status or HbA_2 for accurate diagnosis. These results were almost similar to findings of **Bhushan R et al., (2018)** [23], who found that Mentzer and Ehsani indices were able to correctly diagnose 97.1% and 96.8% cases respectively. **Demir A et al., (2002)** [27] showed that 90% and 92% of the patients were correctly identified with RBC and RDWI, respectively.

LIMITATIONS

Due to the present study's limited sample size and the fact that it was conducted at only one facility, its findings must be confirmed in future longitudinal studies encompassing more hospitals and a wider population.

CONCLUSION

Result of present research leads to conclusion that the percentage of correctly diagnosed patients was highest with RDW followed byMentzer index, RDW and Kerman 1 index. The results of the current study highlight the fact that red cell indices are now reasonably sensitive, specific, reproducible, and exact and can be relied upon for usage in peripheral health clinics without access to more expensive tests like electrophoresis and HPLC. Additionally, it can reduce the costs associated with β-Thalassemia mass screening. Hematologic indicators have been analysed in several research from around the world, and it has been discovered that their performance varies depending on the environment. Therefore, HPLC should be made available at the ground level for screening and early diagnosis in order to prevent any loss of life, whether it to be the mother or foetus, and its impact on lowering maternal mortality as well. HPLC is the gold standard for detection of thalassemia as well as several different hemoglobinopathies, and especially because it can detect abnormal haemoglobin variants with normal blood cell indices which can be missed by routine basic haematological analyzer.

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