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Reliability of Different RBC Indices to Differentiate Between Beta Thalassemia Trait and Iron Deficiency Anemia During Antenatal Screening

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Abstract

Thalassemia and related hemoglobinopathies affect the quantity and quality of haemoglobin. High performance liquid chromatography (HPLC) is considered the gold standard for diagnosis of hemoglobinopathies. This test is not available at many peripheral centers. Many formulae or indices based on complete blood counts are available that can aid in the diagnosis. The aim of the study was to analyze the reliability of these indices in distinguishing between the most common causes of microcytic hypochromic anemia i.e. iron deficiency anemia (IDA) and hemoglobinopathies. A retrospective study was performed on 2000 women who underwent antenatal screening for beta thalassemia trait (β - TT) and related hemoglobinopathies. Nine indices were calculated among both the groups using complete blood count parameters. Sensitivity and specificity of these indices to detect beta -TT versus IDA was compared. Likelihood ratio, accuracy and youden's index for these indices were calculated. Sensitivity of Shine and Lal index was highest (98.4%) followed by Kerman 1 index (66.7%). Mentzer index, England and Fraser index, RDW index and Ehsani index each, showed high specificity (99.66%). Kerman 1 index could point towards the correct diagnosis in 97.5% of the patients. Youden index was highest for Kerman 1 index (65.4) followed by RDW index (63.1). None of the index was 100% sensitive or specific.

*Keywords:*Haemoglobin,RBC indices,High performance liquid chromatography,antenatal screening

Introduction

Thalassemias and related hemoglobinopathies are caused by genetic mutations of the hemoglobin (Hb) genes resulting in reduced production or total absence of one or more globin chains, hence affecting the quantity and the quality of haemoglobin [1]. Ten percent of the total world thalassemics are born in India every year [2].Certain communities in India, like Sindhis, Gujratis, Punjabis, and Bengalis, are more commonly affected with beta thalassemia, the incidence varying from 1 to 17% [3].Diagnosis of beta- thalassaemia trait (β-TT) often becomes difficult due to its overlapping and similar features with iron deficiency anaemia (IDA). There are similarities in red cell indices between the two disorders such as reduced Hb, MCV and MCH. Various electrophoretic approaches, including cellulose acetate electrophoresis, isoelectric focusing in polyacrylamide or agarose gel, as well as high-performance liquid chromatography (HPLC), immunological assays, structural analysis and genotype methods, are used to investigate hemoglobinopathies [4].

High performance liquid chromatography (HPLC) forms an important gold standard tool for screening and detection of various hemoglobinopathies with rapid, reproducible and

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Table 1: Different indices which were studied

INDICES	FORMULAE	β- TT	IDA
RDW	β- TT=<13	<13	>13
MENTZERS	(MCV/RBC)	<13	>13
EFI	MCV-(5XHb)-RBC-3.4	<0	>0
SLI	MCVXMCVXMCH/100	<1530	>1530
GKI	MCVXMCVXRDW/HbX100	<65	>65
SRIVASTAVA	MCH/RBC	<3.8	>3.8
RDW Index	MCVXRDW/RBC	<220	>220
SIRDAH	MCV-RBC-(3Hb)	<27	>27
EHSANI	MCV-(10XRBC)	<15	>15
KERMAN1	MCVXMCH/RBC	<300	>300

precise results [5]. 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 β-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 β-TT or IDA [6]. An ideal discrimination index has high sensitivity and specificity; that is, it can detect the maximum number of patients with β-TT (high sensitivity) while eliminating patients with IDA (high specificity). In this study, we compared the ability of ten indices to distinguish β-TT from IDA by calculating their sensitivity, specificity, and Youden's index values.

Material and Methods

A retrospective study was performed on 2000 women who underwent antenatal screening for β thalassemia trait (β- TT) and related hemoglobinopathies. Erythrocyte microcytosis and hypochromia was assessed using automated blood cell counter Sysmex KX-21 and by peripheral smear. All cases with normal MCV and MCH were not tested further (N=739). All samples which were microcytic (MCV<80fl) and hypochromic (MCH< 27pg) were tested for the serum ferritin levels by ELISA method. The cases with reduced level of serum ferritin were labelled as iron deficiency anemia (N=1194) while those with normal or increased serum ferritin level (N=67) were subjected to HPLC examination. On HPLC examination, there were 59 cases of β-thalassemia trait. Ten indices (Table 1) were calculated among both the groups i.e. β-thalassemia trait (N=59) and iron deficiency anemia (N=1194) using complete blood count parameters. Sensitivity and specificity of these indices to detect β-TT versus IDA was compared. Likelihood ratio, accuracy and Youden's index for these indices were also calculated. 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 [6, 7].

Results

Hemoglobin concentration in the β-TT group was 10.88±2.35g/dl, and that in the IDA group was 8.84 ±1.14 ($P < 0.05$). MCV was 67.02± 5.46fl and MCH was 20.96± 1.99pg in the β-TT group. These values were lower than those in the IDA group (73.83± 3.04 and 24.48± 2.52, respectively; $P < 0.05$). Thus, the amount of microcytosis and hypochromia was more in β-TT than IDA. The 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 ($P < 0.05$) (Table 2).

None of the indices studied showed 100% sensitivity or specificity. However, Shine and Lal index demonstrated highest sensitivity (98.4%) to detect β thalassemia trait but low specificity (8.46%). Kerman 1 index showed second highest sensitivity (66.7%). Mentzer index, England and Fraser Index, RDW index and Ehsani index each, showed high specificity (99.66% each). Moreover, RDW index has highest positive predictive value (90.91%), while Green and King Index have highest negative predictive value (98.87%) (Table 3). Kerman 1 index gave the correct diagnosis in 97.5% of the patients (Table 4). Youden's index showed following ranking with respect to the indices' ability to distinguish between β

Table 2: Different parameters among the cases of thalassemia and iron deficiency anemia

Parameters		AGE(yr)	Hb	RBC	MCV	MCH	RDW
THALASSEMIA CASES	N	63	63	63	63	63	63
	Mean	24.22	10.886	5.625	67.02	20.96	17.2
	Std. Deviation	2.562	2.3528	1.0842	5.461	1.992	3.163
	Median	25	10.6	5.07	67.5	21.2	16.6
	Minimum	19	5.3	3.2	50	17	13
	Maximum	34	15.9	7	77	26	30
IRON DEFICIENCY ANEMIA CASES	N	1187	1187	1187	1187	1187	1187
	Mean	23.89	8.843	3.703	73.83	24.48	16.94
	Std. Deviation	2.596	1.1497	0.2748	3.049	2.525	1.572
	Median	24	8.7	3.7	74.3	25	17
	Minimum	18	6.2	3	60	2	12
	Maximum	34	13.9	4.8	80	27	24
p value		0.324	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.517

thalassemia trait and iron deficiency anemia- highest for Kerman 1 index (65.4%) > RDW index (63.1%) > Green and King index(56.3%) > Mentzer index(42.5%) > Ehsani index(36.1%) > Sirdah index (36%)>Srivastava index (35.5%) > England and Fraser index (25%) > Shine and Lal index (6.8%) > RDW (0.23%) (Table 3) .

Discussion

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 [8].

Shine and Lal index demonstrated highest sensitivity in the present study (98.4%) (Table 3). This was similar to the study done by Boardbar *et al* [9] (87.6%) and Batebi *et al* [10] (83.1%). Bain (1988) reported that Shine and Lal index successfully identified 57 of 58 index pregnancies in patients with beta thalassaemia trait [11]. However, the specificity was low in the present study (8.46%).

Mentzer index in this study showed sensitivity of 42.86% with highest specificity i.e. 99.66% (Table 3). Batebi *et a*l [10] reported sensitivity and specificity of Mentzer index as 86.3% and 85.4% respectively. Some have reported a lower sensitivity of 67% in Mentzer's index while other studies have shown higher sensitivity with this index (82–95%) [11-14].This study showed sensitivity and specificity of RDW as 7.94% and 92.29% respectively (Table 3). Garg *et al* [8] showed specificity of this index as 94.8%.

Another study by Demir *et a*l (2002) showed that Youden's indices of RBC count and RDW were the highest, with values of 82% and 80% respectively [12]. However, in present study, Youden index was highest in Kerman 1 (65.4%) and RDW index (63.1%) (Table 3). AlFadhli *et al* showed that the England and Fraser index had the highest Youden's index value (98%) for correctly differentiating β-TT and IDA, whereas the Shine and Lal index was ineffective [13]. Vehapoglu A *et al* [6] showed that Mentzer index had the highest Youden's index for correctly distinguishing β-TT and IDA (81%).

The England and Fraser and the Shine and Lal indices had the lowest Youden's index values of

Table 3: Sensitiv ity, specificity and other parameters of different indices for detection of β- thalassemia trait

INDEX	Senstivity	Specificity	PPV	NPV	LR+	LR-	Accuracy	Youden's index
RDW	7.94%	92.29%	5.15%	95.00%	1.03	0.99	88	0.23
MENTZERS (MCV/RBC)	42.86%	99.66%	87.10%	97.06%	127.93	0.57	96	42.5
EFI MCV-(5XHb)-RBC-3.4	25.40%	99.66%	80.00%	96.20%	75.81	0.74	95	25
SLI MCVXMCVXMCH/100	98.41%	8.46%	5.37%	98.02%	1.0751	0.18	12	6.8
GKI MCVXMCVXRDW/HbX100	60.32%	96.06%	44.71%	98.87%	15.323	0.41	94	56.3
SRIVASTAVA MCH/RBC	36.51%	98.99%	65.71%	96.73%	36.325	0.64	99	35.5
RDW Index MCVXRDW/RBC	63.49%	99.66%	90.91%	98.10%	189.52	0.36	97	63.1
SIRDAH MCV-RBC-(3Hb)	36.51%	99.58%	82.14%	96.75%	87.181	0.63	96	36
EHSANI MCV-(10XRBC)	36.51%	99.66%	85.19%	96.75%	108.98	0.63	96	36.1
KERMAN1 MCVXMCH/RBC	66.67%	98.83%	75.00%	98.25%	56.857	0.33	97	65.4

51% and 10.2% respectively, in their study. In 2009, Ehsani *et al* showed that the best discrimination index according to Youden's criteria was the Mentzer index (90%), followed by the Ehsani index (85%) [14].

In this study, Mentzer and Ehsani indices were able to correctly diagnose 97.1% and 96.8% cases respectively (Table 4). Ehsani *et al* showed that the Mentzer and Ehsani indices were able to correctly diagnose 94.7% and 92.9% of cases respectively [14].In a study by Madan *et al* (1999), a MCV below 80 fL and a MCH value below 27 pg were found to be very sensitive markers in the detection of β-TT, even in the presence of iron deficiency ($P < 0.0001$) [15].

Present study showed sensitivity and specificity of Green and King index as 60.32% and 96.06% (Youden index, 56.3%) (Table 3). Ntaios *et al* (2007) reported that the Green and King index was the most reliable index, as it had the highest sensitivity (75.06%) and Youden's index (70.86%) for detecting β-TT [16]. A similar result for the Green and King index (Youden's index, 80.9%) was found by Urrechaga *et al* [17].

Ferrara *et al* (2010) demonstrated that RDWI had the highest sensitivity (78.9%), England and Fraser index had the highest specificity and highest Youden's index (0.99 and 0.64, respectively) [18]. The differences in various studies can be due to the inter-population differences in the effectiveness of various RBC indices for discriminating β-TT from IDA and could be

Table 4: Table depicting the correctly diagnosed cases by different RBC indices

INDEX	TT	IDA	CORRECTLY DIAGNOSED (%)
MENTZER β-TT<13 IDA>13	27 32	4 1190	1217 (97.1%)
RDWI β-TT<220 IDA>220	38 21	12 1182	1220 (97.3%)
SHINE AND LAL INDEX β-TT<1530 IDA >1530	58 1	1089 105	163 (13%)
SRIVASTAVA INDEX β-TT<3.8 IDA>3.8	23 36	12 1182	1205 (96.1%)
GREEN AND KING INDEX β-TT<65 IDA>65	38 21	47 1147	1185 (94.5%)
SIRDAH INDEX β-TT<27 IDA>27	23 36	5 1189	1212 (96.7%)
EHSANI β-TT<15 IDA>15	23 36	4 1190	1213 (96.8%)
ENGLAND FRASER INDEX β-TT<0 IDA>0	16 43	4 1190	1206 (96.2%)
RDW β-TT<13 IDA>13	5 54	92 1102	1107 (88.3%)
KERMAN1 β-TT<300 IDA>300	42 17	14 1180	1222 (97.5%)

attributed to differences in the mutation spectrum of the thalassemia disease in different populations [19].

The ability to discriminate IDA from β-TT by different indices also depends on the age of the patient [20] and may be influenced by the pregnant state. The present study was done in pregnant women. Hence, the values may not be representative of the general population and not all indices being used worldwide were included in the present study.

The basic hematological analysis in present study was done on Sysmex KX 21 three part analyzer which cost generally between two to four lakh compared to the HPLC analyzer which costs more than 8 to 9 lakh .Moreover, due to set up issues and technical staff needed, it is practically not possible as for now to have HPLC analysis being available at many of the peripheral centers in a resource poor country. Whereas, three part analyzer is the basic analyzer needed for nearly all baseline hematological assessment of all the patient and all the hematological indices can be calculated using it using designated formulae.

Thus, considering, non availability of automation at the grass root level where most screening is being done, thalassemia indices can be used for screening the patients with low MCV and MCH values. Though HPLC definitely is gold standard for detection of thalassemia as well as several different hemoglobinopathies and especially it can detect abnormal hemoglobin variants with normal blood cell indices which can be missed by routine basic hematological analyzer, it should be made available at the ground level, for screening as well as to make early diagnosis so as to prevent any loss of life, of mother or fetus and its impact on lowering maternal mortality as well as mortality and morbidity in the general population.

Conclusion

In the present study, Shine and Lal index demonstrated highest sensitivity (98.4%). Mentzer index, England and Fraser index, RDW index and Ehsani index each, showed highest specificity (99.66% each). RDW index has highest positive predictive value (90.91%), while Green and King index has highest negative predictive value (98.87%). Youden index which tells about the overall performance of a diagnostic test was highest for Kerman 1 index (65.4%).

According to our results, the percentage of correctly diagnosed patients was highest with Kerman 1 index (97.5%) followed by RDW index (97.3%) and Mentzer index (97.1%). From the present study, we can emphasize that with the easier availability of basic automation in haematology, red cell indices have become fairly sensitive, specific, reproducible and precise and can be reliably used in the peripheral health centers where expensive investigations like electrophoresis and HPLC are not available. Moreover, it can minimize the expenditure during mass screening of β -TT. However, it should be kept in mind that several studies have shown that none of the red cell indices carry 100% sensitivity and specificity in diagnosing β -TT, but adequate utilization of combination of these indices can facilitate identification of the majority of β -TT cases at no additional cost to the healthcare system which is important in the developing country like India till HPLC or chromatography is available to all at the grass root level.

Authors' Contribution

None

Conflict of Interests

None

Ethical Considerations

None

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