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Evaluation of the Discrimination between Beta-Thalassemia Trait and Iron Deficiency Anemia Using Different Indexes

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Abstract

Background: Iron deficiency anemia (IDA) and beta-thalassemia trait (BTT) are the most common types of microcytic hypochromic anemias. The aim of this study was to evaluate the reliability of different RBC indices for discrimination between IDA and BTT in Sudanese patients.

Methods and Results: This cross-sectional laboratory-based study was conducted among 200 patients (100 patients suffering from BTT and 100 from IDA) who attended the public health hospitals of Khartoum State (Sudan), from Jan 2021 to Feb 2022. The diagnosis of BTT was based on CBC and hemoglobin electrophoresis, and was confirmed by PCR. The diagnosis of IDA was based on complete blood count, reduced serum iron levels, and ferritin levels. Pregnant women, patients who had received a blood transfusion within three months of the study, and patients with thalassemia coexisting with IDA or other hemoglobinopathies were excluded from the study. A series of red blood cell indices were analyzed to differentiate IDA and BTT. The sensitivity, specificity, positive predictive value, negative predictive value, and Youden index (*J*) were calculated for each index.

In BTT and IDA patients, mean values of Hb, hematocrit, and mean corpuscular volume were 10.9g/dL, 33.96%, 59.11fL, and 8.5g/dL, 26%, 68fL, respectively. All RBC indices were decreased in BTT and IDA. Mean RBC count was increased in BTT while showing normal values in IDA. In BTT patients, hemoglobin electrophoresis showed high HbA_2 (6.4%) and HbF (1.95%) but a decreased HbA (78.2%).

Conclusion: The best discrimination index according to *J* was Mentzer index (0.85), followed by Sidrah index (0.83), Ehsani index (0.81), RBC count (0.80), RDWI (0.79), Green and King index (0.76), MDHL (o.76), Srivastava index (o.76), and England and Fraser index (0.7). The lowest *J* was presented in Ricerca index (0.45), Shine and Lal index (0.01), and MCHD (0). (**International Journal of Biomedicine. 2022;12(3):375-379.**).

Keywords: beta-thalassemia trait • iron deficiency anemia • red blood cell indices

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Abbreviations

BTT, beta-thalassemia trait; **CBC**, complete blood count; **EI**, Ehsani index; **EFI**, England and Fraser index; **GKI,** Green and King index; **Hb**, hemoglobin; **HbF**, fetal hemoglobin; **HbA**, adult hemoglobin; **HCT**, hematocrit; **IDA**, iron deficiency anemia; **RBC,** red blood cell; **MCV,** mean corpuscular volume; **MCHC,** mean corpuscular hemoglobin concentration; **MCH**, mean corpuscular hemoglobin; **MDHL**, mean density of Hb per liter of blood; **MCHD:** mean cell Hb density;. **MI,** Mentzer index; **NPV**, negative predictive value; **PPV**, positive predictive value; **RDWI**, RBC distribution width index; **RI**, Ricerca index; **SiI**, Sidrah index; **SrI**, Srivastava index; **SLI**, Shine and Lal index; **TIBC**, total iron binding capacity; ***J***, Youden index.

Introduction

Iron deficiency anemia (IDA) and beta-thalassemia trait (BTT) are the most common types of microcytic hypochromic anemias. Microcytic anemia, characterized by smaller than normal RBCs, is caused by iron deficiency, inflammatory disease, and thalassemia.[1] The most common microcytic anemia in Sudan is IDA, with a prevalence of 23.46% to 76%.[2] Most patients are diagnosed on routine blood examination.[3] IDA resulting from a lack of sufficient iron to synthesize hemoglobin is the most common hematological disease in infants and children. IDA is counted as the foremost common cause of hypochromic microcytic anemia, and the most common reason behind this sort of anemia is decreased iron reserves in the body.[4] The World Health Organization estimates that approximately half of the 1.62 billion cases of anemia worldwide are due to iron deficiency.[5] The most common causes of IDA are blood loss, reduced absorption of iron, menstrual blood loss, malabsorption, and epistaxis.[6]

Thalassemia is a group of genetically determined blood disorders characterized by microcytic, hypochromic anemia resulting from a decreased synthesis of alpha or beta chains of hemoglobin (Hb).[7] Gene frequencies for the α- and β-thalassemia on a global basis range from 1% to more than 80% in areas where malaria is endemic.[8-9]

Beta-thalassemia results from point mutations in the beta-globin gene. Beta-thalassemia includes three main types based on the zygosity of the beta-gene mutation: (a) major thalassemia known as Cooley's anemia or Mediterranean anemia, caused by a homozygous mutation (beta-zero thalassemia) of the beta-globin gene, resulting in the total absence of beta chains; (b) thalassemia intermediate (the condition between major and minor beta-thalassemia) and (c) thalassemia minor, also called "beta-thalassemia carrier," "beta-thalassemia trait" or "heterozygous beta-thalassemia," caused by a heterozygous mutation (beta-plus thalassemia) in which beta chains are underproduced.

Major beta-thalassemia occurs between six and 24 months. Affected infants fail to thrive and become progressively more pale. They may experience recurrent bouts of fever, irritability, feeding problems, diarrhea, growth and bone abnormalities, and progressive enlargement of the abdomen caused by splenomegaly.[8,9] Major beta-thalassemia (Cooley's anemia) is one of the common monogenic hereditary hemoglobin disorders with a widespread geographical distribution. It has been estimated that about 80–90 million people (1.5% of the global population) are carriers of beta-thalassemia, with about 60,000 symptomatic individuals born annually, the great majority in the developing world.[9,10]

Microcytic hypochromic anemia is diagnosed by CBC, including RBC indices and peripheral smear findings, and with other confirmatory tests, such as iron profiles, hemoglobin electrophoresis, and molecular study to detect thalassemia.[1,8] Microcytosis and hypochromia are common presentations of both IDA and BTT. Since the other cause of microcytic anemia is iron deficiency, it is important to differentiate it from BTT. These two conditions cannot be differentiated based on peripheral blood picture because both of these conditions present with decreased Hb, RBC indices, and normal-to-increased RDWI.[1,8] BTT and IDA can be differentiated effectively by involving a battery of tests, including serum ferritin, serum iron, and HbA_2 level estimation. BTT characteristically has increased HbA_2 (4-8%) with variably normal-to-low elevations of HbF.[11]

A beta-thalassemia carrier is commonly not diagnosed until adolescence or adult life. It may be detected using hematological screening examinations and requires expensive diagnosis, which is only available in certain hospitals. Therefore, to avoid much more expensive, time-consuming, and complicated procedures for diagnosic discrimination between thalassemia and other types of microcytic hypochromic anemia, developing easily examined parameters is crucial as they help assess thalassemia carrier diagnosis and avoid misdiagnosis between BTT and IDA.[7]

A definitive, differential diagnosis between BTT and IDA is based on the result of HbA2 electrophoresis, iron profiles, and molecular analysis.[12] To avoid much more expensive, time-consuming, and complicated procedures for discrimination between these disorders, different methods of discrimination between BTT and IDA are employed.[13-20]

The aim of this study was to evaluate the reliability of different RBC indices for discrimination between IDA and BTT in Sudanese patients.

Materials and Methods

This cross-sectional laboratory-based study was conducted among 200 patients (100 patients suffering from BTT and 100 from IDA) who attended the public health hospitals of Khartoum State (Sudan), from Jan 2021 to Feb 2022. The diagnosis of BTT was based on CBC and hemoglobin electrophoresis, and was confirmed by PCR. The diagnosis of IDA was based on CBC, reduced serum iron levels, and ferritin levels. Pregnant women, patients who had received a blood transfusion within three months of the study, and patients with thalassemia coexisting with IDA or other hemoglobinopathies were excluded from the study. A series of RBC indices were analyzed to differentiate IDA and BTT (Table 1). The sensitivity, specificity, PPV, NPV, and Youden index (*J)* were calculated for each index.[21]

Table 1.
RBC indices analyzed in the study.

Hematological index	Year	Formula
Mentzer index (MI) [14]	1973	MCV/RBC count
Srivastava index (SrI) [16]	1973	MCH/RBC count
England and Fraser (EFI) [13]	1973	MCV − (5 × Hb) − RBC − 3.4
Shine and Lal (SLI) [15]	1977	MCV × MCV × MCH/100
RDWI	1987	MCV × RDW/RBC
Ricerca index (RI) [18]	1987	RDW/RBC
Green and King (GKI) [20]	1989	MCV × MCV × RDW/ (Hb × 100)
MDHL	1999	(MCH/MCV) × RBC count
MCHD	1999	MCH/MCV
Sirdah index (SiI) [19]	2008	MCV − RBC − (3×Hb)
Ehsani index (EI) [17]	2009	MCV − (10×RBC)

Table 2

Hematological findings of patients with BTT.

Statistics	Hb, g/dL	HCT, %	RBC count, $\times 10^6$/L	MCV, fL	MCH, pg/cell	MCHC, g/dL	RDWI, %	HbA_2 ,%	HbF, %	HbA, %
Mean	10.9	33.96	5.77	59.11	19.1	32.18	18.456	6.4	1.948	78.29
Minimum	6.8	23.4	4.1	44.2	13.3	27	12.5	3.6	0.4	70.1
Maximum	15	46	7.5	81.8	31.8	36.6	28.9	12	10.4	87.1
SD	1.82	4.964	0.81	6.52	2.931	1.751	2.8235	1.5	1.848	3.076

Table 3.

Hematological findings of patients with IDA.

Statistics	Hb, g/dL	RBC count, $\times 10^6$/L	HCT, %	MCV, fL	MCH, pg/cell	MCHC, g/dL	RDWI, %	Serum iron, µg/dL
Mean	8.5	3.9	26	68	22.2	33	17.7	32
Minimum	4.9	2	15	44	12.7	26	11.7	13
Maximum	12	5.3	37	79	28.4	39	38.1	42
SD	2.1	0.8	5.8	7.4	3.27	2.1	4.36	

Results and Discussion

All CBC results represented microcytic hypochromic anemia. In BTT and IDA patients, mean values of Hb, HCT, and MCV were 10.9g/dL, 33.96%, 59.11fL, and 8.5g/dL, 26%, 68fL, respectively. All RBC indices were decreased in BTT and IDA (Tables 2 and 3). Mean RBC count was increased in BTT while showing normal values in IDA. In BTT patients, hemoglobin electrophoresis showed high HbA_2 (6.4%) and HbF (1.95%) but a decreased HbA (78.2%).

Sensitivity, specificity, and *J* were calculated to evaluate the reliability of 12 RBC indexes in differentiating between BTT and IDA (Tables 4-7).

The results obtained showed that both IDA and BTT are characterized by classical features of microcytic hypochromic anemia according to CBC (decreased Hb, HCT, MCV, MCH, and MCHC) and blood picture. Moreover, BTT shows a significant increase in HbA_2 and HbF.

The best discrimination index, according to Youden index (*J*), was Mentzer index (MI),[14] followed by Sidrah index (SiI),[19] Ehsani index (EI),[17] RBC count, RBC distribution width index (RDWI), Green and King index (GKI),[20] mean density of Hb per liter of blood (MDHL), Srivastava index (SrI),[16] and EFI (England and Fraser index),[13] and the lowest *J* was presented in Ricerca index (RI),[18] Shine and Lal index (SLI),[15] and mean cell Hb density (MCHD). On the other hand, the highest sensitivity values were obtained with MI, followed by EI,[17] RBC count/SiI, and RDWI. The rest of the indexes obtained less than 80%.

The high specificity index and a low sensitivity index for BTT were obtained by EFI, followed by SiI and MDHL. The most reliable index was MI, which obtained higher *J*, sensitivity, and NPV and seemed to be an appropriate index for screening. The second most reliable was SI, which obtained a higher *J*, specificity, and positive predictive value. The third index was EI, which obtained high *J*, sensitivity, and negative predictive value. The fourth most reliable index was obtained by RBC count and RDWI. In this study, SLI, RI, and MCHD indexes were not valid since they have low values for discrimination.

MI was found to be more reliable in detecting true positive cases of BTT and IDA (90% and 95%, respectively), whereas it was more specific to picking the true negative cases of BTT and IDA with a specificity of 95% and 90%, respectively. *J*, which is the most reliable method to measure the validity of hematological indices and formulas, showed a higher value (0.85). This result correlates with a previous study performed by Alam et al.,[22] which reported that MI has good validity; the sensitivity and specificity were 93% and 84%, respectively, with an AUC of 91.9%, indicating that MI was valid for use as a screening tool. On the other hand, Ferrara et al.[23] showed that the highest sensitivity was obtained with RDWI (78.9%) , the highest specificity and *J* with EFI (99.1 and 64.2%, respectively), and the highest positive and negative prognostic value (80.2%) with GKI. Shreya Bose[10] showed that MI proved to be a reliable tool in differentiation, with sensitivity and specificity (90% and 85%, respectively) for IDA and (85% and 90%, respectively) for BTT, respectively; *J* was significant, with a value of 75%. Ehsani et al. [17] showed that the best discrimination index according to *J* was MI (90.1%), followed by EI (85.5%); MI and EI were able to correctly diagnose 94.7% and 92.9% of cases, respectively, which was in agreement with our study.

On the other hand, our results disagree with the data obtained by Batebi et al.[24] The authors showed that EFI, with its higher sensitivity (87.2%), seems to be an appropriate index for screening. SLI, with a coefficient of 0.738, has the highest correlation with final definite diagnosis tests.

There are varying results obtained in different studies. More extensive studies are needed to establish the optimal discrimination index and confirm the results obtained in this process and help select appropriate individuals for a more detailed examination

Table 4.
Sensitivity and specificity of indexes between BTT and IDA.

Index	Cut off	BTT (n)	IDA (n)	Sensitivity (%)	Specificity (%)
MI	<13	90	5	90	95
	>13	10	95	95	90
SLI	<1530	98	99	98	1
	>1530	2	1	1	98
EFI	< 0	70	0	70	100
	> 0	30	100	100	70
RDWI	<220	83	4	83	96
	>220	17	96	96	83
GKI	<65	79	3	79	97
	>65	21	97	97	79
EI	<15	88	7	88	93
	>15	12	93	93	88
SrI	<3.8	79	3	79	97
	>3.8	21	97	97	79
SiI	<27	85	2	85	98
	>27	15	98	98	85
RI	<4.4	93	48	93	52
	>4.4	7	52	52	93
MHDL	>1.63	78	2	78	98
	<1.63	22	98	98	78
MCHD	>0.3045	88	88	88	12
	<0.3045	12	12	12	88
RBC count	>5	85	5	85	95
	<5	15	95	95	85

Sensitivity (Se) = [true positive/(true positive + false negative)] ×100.
Specificity (Sp) = [true negative/(true negative + false positive)] ×100.

Table 5.
Reliability of study indexes for diagnosis BTT and IDA.

Index	Cut off	NPV,%	PPV,%	*J*
MI	<13	90.5	94.7	0.85
	>13	94.7	90.5	
SLI	<1530	33.3	49.7	0.01
	>1530	49.7	33.3	
EFI	< 0	76.9	100	0.7
	> 0	100	76.9	
RDWI	<220	85	95.4	0.79
	>220	95.4	85	
GKI	<65	82.2	96.3	0.76
	>65	96.3	82.2	
EI	<15	88.6	92.6	0.81
	>15	92.6	88.6	
SrI	>3.8	82.2	96.3	0.76
	<3.8	96.3	82.2	
SiI	<27	86.7	97.7	0.83
	>27	97.7	86.7	
RI	<4.4	88.1	66	0.45
	>4.4	66	88.1	
MDHL	>1.63	81.7	97.5	0.76
	<1.63	97.5	81.7	
MCHD	>3045	50	50	0
	<3045	50	50	
RBC count	>5	86.4	94.4	0.8
	< 5	94.4	86.4	

PPV = true positive / (true positive + false positive) ×100.
NPV = true negative/ (true negative + false negative) ×100.
The Youden index (J) was defined as [25]:

J = sensitivity + specificity −1, where sensitivity and specificity are expressed in proportion.

The maximum value of J is 1 (perfect test) and the minimum is 0 when the test has no diagnostic value.

Table 6.
Descriptive statistics for studied indices in patients with BTT.

	MI	SLI	EFI	RDWI	GKI	EI	SrI	SiI	RI	M-L	M-D
Mean	10.5	692.4	-4.72	191.72	59.98	1.46	3.39	20.55	3.25	1.86	0.32
Min	6.59	265.7	-24.6	113.24	39.24	-24.6	2.03	6.3	2.32	1.25	0.27
Max	17.76	1813.3	17.7	352.58	129.56	33.4	6.36	37.3	5.78	2.48	0.495
SD	2.30	267.6	9.58	44.33	16.29	11.84	0.83	7.22	0.69	0.30	0.029

Min, minimum; Max, maximum; M-L, MDHL; M-D, MCHD

Table 7.
Descriptive statistics for study indices in patients with IDA.

	MI	SLI	EFI	RDWI	GKI	EI	SrI	SiI	RI	M-L	M-D
Mean	18	1060.97	18.0	321.69	99.19	29.12	6.04	38.47	4.76	1.25	0.325
Min	11	244.76	2.08	199.95	59.71	3.2	2.79	25.33	3.05	0.72	0.261
Max	37	1530.06	42.5	818.09	231.59	53.8	13.25	56.1	11.82	1.69	0.386
SD	5.4	315.81	8.97	118.47	33.03	11.06	2.07	6.91	1.91	0.239	0.022

Min, minimum; Max, maximum; M-L, MDHL; M-D, MCHD

In conclusion, the best discrimination index according to *J* was Mentzer index (0.85), followed by Sidrah index (0.83), Ehsani index (0.81), RBC count (0.80), RDWI (0.79), Green and King index (0.76), MDHL (o.76), Srivastava index (o.76), and England and Fraser index (0.7). The lowest *J* was presented in Ricerca index (0.45), Shine and Lal index (0.01), and MCHD (0). These indices have many advantages: simple, fast, and inexpensive.

Competing Interests

The authors declare that they have no competing interests.

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