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Validity of Erythrocyte Indices in Differentiation between Iron Deficiency Anemia and β-Thalassemia Trait in Children

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Abstract

Iron deficiency anemia (IDA) and β-thalassemia trait (BTT) are the most common causes of hypochromic microcytic anemia (HMA). Various erythrocyte indices that may help in the initial discrimination between IDA and BTT have been reported, but data evaluating their reliability in children are scarce. We aimed to evaluate the validity of 12 erythrocyte indices in the differentiation between IDA and BTT in children. These indices were red blood cell (RBC) count, Mentzer Index, England and Fraser Index, Srivastava Index, Shine and Lal Index, RBC distribution width (RDW), Ricerca Index, Green and King Index, RDW Index, Sirdah Index, Ehsani Index, and Serdar Index. Among 1,444 children with HMA, 136 (9.4%) were stratified into the IDA group and 137 (9.5%) into the BTT group. Of the 12 indices, the Green and King Index showed the highest reliability, as it had the highest Youden's index (75.1%). Its sensitivity, specificity, positive predictive value, negative predictive value and correct diagnosis rate were 92.7%, 82.4%, 84.1%, 91.8% and 87.5%, respectively. The second most reliable index was the RDW Index, having a Youden's index, sensitivity, specificity, positive predictive value, negative predictive value and correct diagnosis rate of 64%, 94.2%, 69.9%, 75.9%, 92.2% and 82%, respectively. Receiver operating characteristic analysis showed that the revised cut-off values for the Green and King Index and RDW Index had higher sensitivity and specificity levels than the cut-off values commonly used in the literature. The findings of this study suggest the superiority of the Green and King Index and the RDW Index as screening tools in the initial differentiation between IDA and BTT among children with HMA.

Keywords: Iron deficiency anemia, β-thalassemia trait, hypochromic microcytic anemia, erythrocyte indices, discrimination index

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Introduction

Iron deficiency anemia (IDA) is a prevalent problem worldwide, mainly affecting populations in underdeveloped and developing countries. It is the most common cause of hypochromic microcytic anemia (HMA). Beta-thalassemia trait (BTT), another common cause of HMA, is prevalent in certain regions of the world.[1] It has a prevalence of 2.5% in the Isparta province of Turkey. The city of Isparta is located in the Mediterranean region of Turkey, and its distance from Antalya is approximately 130 km.[2]

The correct diagnosis of a patient with HMA is important in several aspects. Identification of BTT is particularly crucial for genetic counseling, whereas IDA as the underlying cause warrants appropriate therapy. In most affected children, IDA can easily be treated with oral iron supplementation and dietary advice and may be associated with adverse neurocognitive outcomes if left untreated. On the other hand, blanket therapy with iron supplements in a BTT case may lead to excessive iron intake with possible harmful effects. A recent randomized controlled trial study including 562 children reported that adolescents who had no anemia and received iron supplements in infancy showed poorer performance on visual-motor and quantitative reasoning and displayed more errors on neurocognitive tasks than those who had no anemia and did not receive iron supplements in infancy.[3]

On competitive blood count (CBC) testing, IDA and BTT cases usually have similar hematologic findings, such as decreased mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH), and it may be quite challenging to discriminate between the two conditions correctly. The diagnosis of IDA usually relies on a low serum ferritin level and/or transferrin saturation index (TSI), whereas BTT is mostly diagnosed by an increased hemoglobin (Hb) A_2 (HbA_2) level in Hb electrophoresis. However, these laboratory investigations have significant costs and are time-consuming. In addition, they are not accessible to a large number of physicians. Since the 1970's, several indices such as the Mentzer Index calculated from simple erythrocyte parameters have been proposed for the inexpensive and simple differentiation between IDA and thalassemia carrier status.[4-15]

Most studies evaluating the efficiency of various erythrocyte indices in discriminating between IDA and BTT have been conducted in adults, and data in children are scarce.[16-26] We aimed to evaluate the reliability of 12 erythrocyte indices in the differential diagnosis between IDA and BTT in children with HMA.

Material and Method

This study included children aged 6 months-18 years who admitted to a pediatric outpatient clinic of Süleyman Demirel University, Medical Faculty Hospital between January 2018 and January 2020, and were found to have HMA using the data obtained from electronic records of the hospital. HMA was defined as follows: Hb < mean for age and sex-2 standard deviations (SD) (6 months-2 years <10.5 g/dL, 2-12 years <11.5 g/dL, boys aged 12-18 years <13 g/dL and girls aged 12-18 years <12 g/dL) and MCH < mean for age and sex-2 SD (6 months-2 years <23 pg, 2-6 years <24 pg, 6-18 years <25 pg) and MCV < mean for age and sex-2 SD (6 months-2 years <70 fL, 2-6 years <75 fL, 6-12 years <77 fL and 12-18 years <78 fL).[27] If the case had more than one CBC result consistent with HMA during the mentioned two-year period, then only the first testing was was taken into account.

Children with HMA were stratified into three groups as follows: 1) those with a serum ferritin level <12 ng/mL or those without a ferritin level measured, but with a TSI level <10% were classified in IDA group, 2) children having an HbA_2 level ≥3.5% in Hb electrophoresis were included in the BTT group; and 3) cases fulfilling the criteria of both IDA and BTT were classified in IDA + BTT group.

The efficiency of the 12 erythrocyte indices in differentiating between IDA and BTT was assessed using the data of children in the IDA and BTT groups, and cases in the IDA+BTT group were excluded from the analyses. The formulas for the erythrocyte indices and their previously proposed cut-off values are presented in **Table 1**.[4-15]

Statistical Analysis

Data were analyzed using the Statistical Package for Social Sciences, version 23.0 (SPSS, Chicago, IL, USA), and for each erythrocyte index, the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), correct diagnosis rate, and Youden's index (YI, sensitivity + specificity -1) were calculated. YI provides a reliable measure of the diagnostic validity of a certain technique because its formula involves both sensitivity and specificity.[28,29] Its value ranges between 0 and 1, and the minimum point for having an acceptable YI is 0.5. Receiver operating characteristic (ROC) analysis was performed to evaluate the erythrocyte indices in the differentiation between IDA and BTT. A p value <0.05 was considered statistically significant.

Süleyman Demirel University Faculty of Medicine Clinical Research Ethics Committee approval (February 2021 and 72 session number) was obtained before starting the study.

Results

During the study period, 1,444 children were found to have at least one CBC finding consistent with HMA. Among them, 136 cases (9.4%; 51 males and 85 females) were included in the IDA group and 137 cases (9.5%; 74 males and 63 females) in the BTT group. Data of nine subjects with both IDA and BTT were not included in the analyses.

Table 2 shows the laboratory findings of the subjects in the IDA and BTT groups, and **Table 3** displays the sensitivity, specificity, PPV, NPV, correct diagnosis rate, and YI of the 12 erythrocyte indices in differentiating BTT from IDA. The Green and King Index and RBC distribution width (RDW) Index had the highest YI (75.1% and 64.0%, respectively) and correct diagnosis rate (87.5% and 82.0%, respectively) (**Table 3**).

In ROC analysis, the revised cut-off values for the Green and King Index and RDW Index were found to have higher levels of sensitivity and specificity than the cut-off values commonly used in the literature. **Table 4** shows the ROC analysis results and the sensitivity and specificity levels of the indices according to the revised cut-off values. **Figure 1** presents the ROC curve.

Discussion

The findings of this study suggest that the Green and King Index and RDW Index may be superior to other erythrocyte indices in differentiation between IDA and BTT cases in our region. Many of the indices previously defined in the literature do not seem to be suitable for our study population.

Among the 1,444 children found to have HMA in the present study, only 282 (19.5%) could be included in the IDA, BTT or IDA + BTT group, and interestingly, the number of cases in the IDA and BTT groups was almost the same. We would rather expect iron deficiency to be the main cause of HMA in our patients. These findings may be explained by the lack of serum iron parameters or the presence of an accompanying infectious or inflammatory state when blood samples were taken, which may have caused an increase in serum ferritin levels in some cases with IDA. In children with HMA who do not fit into IDA diagnosis using markers such as serum ferritin at presentation, serum ferritin and other iron parameters should be examined intermittently in order not to miss IDA diagnosis.

We included only children with HMA in our study. In an individual, iron deficiency may be present without accompanying anemia. In addition, in its early stages, anemia associated with iron deficiency may be normocytic.[30] Cases of iron deficiency unaccompanied by HMA were not included in our study. In addition, we used "12 ng/mL" and "10%" as the cut-off values for low serum ferritin and TSI levels, respectively, which are both below the levels ("20 ng/mL" and "16%", respectively) in some studies in the literature.[18,20,22,23] Therefore, cases with HMA and a serum ferritin level in the range of 12-20 ng/mL or a TSI level in the range of 10-16% were not included in the IDA group in our study. However, the use of such low ferritin and TSI levels may have enabled a relatively high specificity of these variables for IDA diagnosis in our cases with HMA. Finally, as our hospital is the only tertiary care center in our province, most cases with suspected BTT may have been referred to our hospital for further analyses, resulting in a relatively high percentage of children with BTT in our study.

Highlights

- Iron deficiency anemia (IDA) and β-thalassemia trait (BTT) are the most common forms of hypochromic microcytic anemias, and differentiation between these two conditions is important as their management differs completely.
- Several indices calculated from some erythrocyte parameters on hemogram test have been proposed so far for the initial differentiation between IDA and BTT.
- None of these indices is 100% reliable in the discrimination between IDA and BTT.
- In children from Isparta province of Turkey, Green and King Index and red cell distribution width Index (RDWI) may be helpful in the initial differentiation between IDA and BTT.

Iron deficiency accompanying BTT may be thought to interfere with BTT diagnosis by decreasing HbA_2 levels. However, iron deficiency, even when very severe, is very unlikely to significantly interfere with HbA_2-based identification of BTT.[31,32] In patients with iron deficiency, HbA_2 levels have been reported to be lower, but not below the 3.5% cut-off.[33,34] Therefore, we believe that cases with IDA and an HbA_2 level <3.5% in our study are very unlikely to have additional BTT.

Among the erythrocyte indices evaluated here, we found the YI of only four (Green and King Index, RDW Index, Srivastava Index and Sirdah Index) to be acceptable (>50%) in differentiation between IDA and BTT (**Table 3**). Mentzer Index (MCV/RBC), one of the best-known erythrocyte indices in differentiation between IDA and BTT, had a sensitivity of 93.4% in the present study; however, its YI was found to be quite low (40.5%), which can be attributed to its low specificity (47.1%) (**Table 3**). Similar to our findings, some previous studies have also reported a high sensitivity and NPV but a low specificity and PPV of the Mentzer Index for BTT diagnosis.[23,24,26]

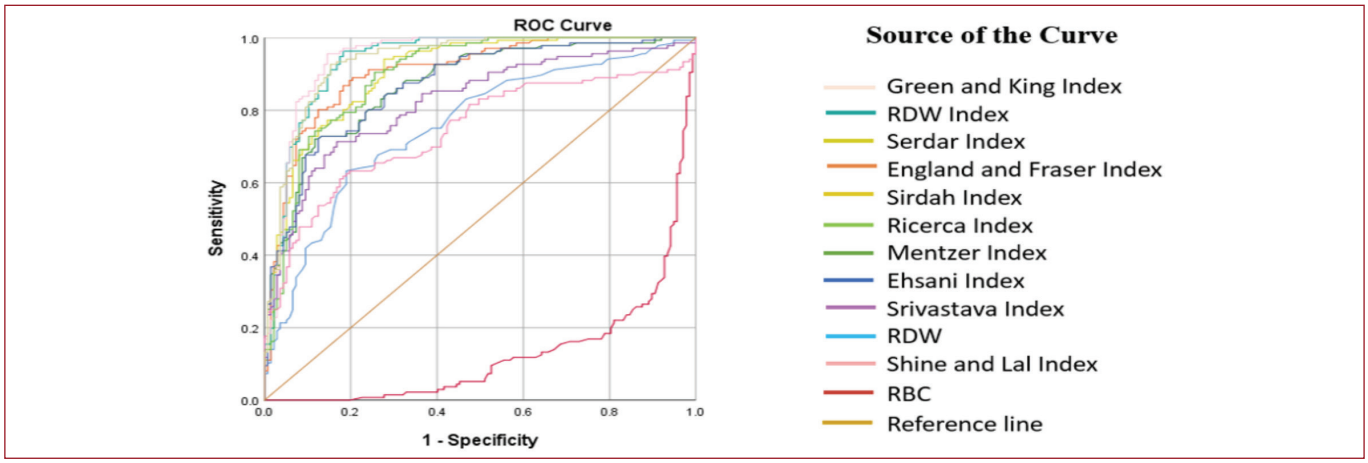

Figure 1. ROC curve of erythrocyte indices for differentiation of BTT from IDA.

ROC; Receiver operating characteristic, BTT; β-thalassemia trait, IDA; Iron deficiency anemia

However, the results of some other studies seem to contradict these observations.[16,18,20-22]

Another erythrocyte index commonly used in differentiation between IDA and BTT is the RBC count (**Table 1**). However, Aslan and Altay[35] reported that in 36 (26%) of 140 children aged 6 to 48 months who had IDA and no accompanying thalassemia trait, the RBC count was high (≥5.0 x 10^6/L) at the time of diagnosis. The authors observed the elevation in RBC count to be more pronounced in patients with mild anemia, who have the highest probability of being misdiagnosed as having a thalassemia trait, and concluded that an elevated RBC count is not reliable in differentiation between IDA and thalassemia trait in certain patient groups. We also found that the RBC count not to be reliable in differentiation between IDA and BTT. Similar to the Mentzer Index, it had low specificity and PPV levels, and its YI was below the acceptable range (**Table 3**).

In a recent study evaluating the roles of 30 erythrocyte indices in differentiation between IDA and BTT in adults, a newly proposed index (Serdar Index) was found to be superior to 29 previously reported indices.[15] However, in our study, which included only children, this new index did not reliably differentiate between IDA and BTT (**Table 3**).

The results of different studies evaluating the efficiencies of various erythrocyte indices in the differential diagnosis of IDA and BTT seem to be controversial.[15-26,35-39] In our study, the Green and King Index and RDW Index were found to be the most reliable indices. Some previous studies from different countries have also reported the Green and King Index to be among the most reliable erythrocyte indices in differentiation between IDA and BTT.[23,32-35] The YI of the Green and King Index ranged between 68% and 79.8% in those studies. In addition, the RDW Index has been found to be among the most valuable indices in the differentiation between IDA and BTT in some previous studies.[23,26,36,39] We also observed the Green and King Index and RDW Index to better differentiate BTT from IDA by using their revised cut-off values found in the ROC analysis (**Table 4**). The Green and King Index was also superior to other indices in ROC analysis (**Table 4**, **Figure 1**).

On the contrary, other studies, including one study from the East Thrace region of Turkey, have reported that the

Table 1. *Formulas and cut-off values of erythrocyte indices used for differentiating IDA and BTT*

Index	Formula cut-off value
RBC count	RBC
BTT	>5
IDA	<5
Mentzer index	MCV/RBC
BTT	<13
IDA	>13
England and Fraser index	MCV-RBC-(5 x Hb)-8.4
BTT	<0
IDA	>0
Srivastava index	MCH/RBC
BTT	<3.8
IDA	>3.8
Shine and Lal index	MCV²xMCH/100
BTT	<1530
IDA	>1530
RDW	RDW
BTT	<14
IDA	>14
Ricerca index	RDW/RBC
BTT	<4.4
IDA	>4.4
Green and King index	MCV² x RDW/(Hb x 100)
BTT	<65
IDA	>65
RDW index	MCV RDW/RBC
BTT	<220
IDA	>220
Sirdah index	MCV-RBC-(3 x Hb)
BTT	<27
IDA	>27
Ehsani index	MCV-(10 x RBC)
BTT	<15
IDA	>15
Serdar index	(MCV² × RDW)/[(10 × RBC)² × MCHC]
BTT	<0.96
IDA	>0.96

BTT; β-thalassemia trait, Hb; Hemoglobin, IDA; Iron deficiency anemia, MCH; Mean corpuscular Hb, MCHC; Mean corpuscular Hb concentration, MCV; Mean corpuscular volume, RBC; Red blood cell, RDW; Red cell distribution width

Table 2. *Hematologic data of the subjects in the IDA and BTT groups*

	IDA group (n=136) Mean ± SD (Min.-Max.)	BTT group (n=137) Mean ± SD (Min.-Max.)
Hb, g/dL	9.82±1.50 (6.2-11.6)	11.15±0.81 (9.8-13.6)
Hct, %	31.20±3.62 (23.3-35.8)	34.32±2.78 (28.3-42.0)
RBC, x10^6/mm^3	5.16±0.44 (4.33-5.89)	5.84±0.48 (4.43-6.88)
MCV, fL	60.57±5.81 (49.6-72.5)	58.54±4.06 (51.5-70.9)
MCH, pg	19.05±2.55 (13.4-23.7)	19.10±1.29 (16.3-22.6)
MCHC, g/dL	31.47±1.50 (26.9-33.6)	32.63±1.00 (30.9-36.1)
RDW, %	19.99±3.61 (13.6-30.0)	16.80±1.70 (14.5-25.1)
HbA2, %	2.37±0.44 (1.3-3.2)†	5.24±0.68 (3.7-6.9)
Ferritin, ng/mL	5.24±2.47 (0.5-11.0)	66.19±117.30 (12.6-780.0)‡
TSI, %	4.28±2.39 (0.25-19.88)	24.04±10.53 (2.27-44.92)‡

†HbA2 was examined in 37 subjects in the IDA group.
‡Serum ferritin and TSI levels were available in 123 and 72 subjects in the BTT group, respectively.
BTT; β-thalassemia trait, Hb; Hemoglobin, Hct; Hematocrit, IDA; Iron deficiency anemia, Max.; Maximum, MCH; Mean corpuscular Hb, MCHC; Mean corpuscular Hb concentration, MCV; Mean corpuscular volume, Min.; Minimum, RBC; Red blood cell, RDW; RBC distribution width, SD; Standard deviation, TSI; Transferrin saturation index

Table 3. *Predictive values of erythrocyte indices for differentiating BTT from IDA*

Index	Sen. (%)	Spe. (%)	PPV (%)	NPV (%)	Corr Dia (%)	YI
Ehsani index	94.2	47.1	64.2	88.9	70.7	41.2
England and Fraser index	98.5	36.0	60.8	96.1	67.4	34.6
Green and King index	92.7	82.4	84.1	91.8	87.5	75.1
Mentzer index	93.4	47.1	64.0	87.7	70.3	40.5
RBC	96.4	44.1	63.5	92.3	70.4	40.5
RDW	0	99.3	0	49.6	49.5	-0.7
RDW index	94.2	69.9	75.9	92.2	82.0	64.0
Ricerca index	99.3	14.0	53.8	95.0	56.8	13.2
Serdar index	97.1	42.7	63.0	93.6	70.0	39.7
Shine and Lal index	100.0	0	50.2	0	50.2	0
Sirdah index	94.2	59.6	70.1	91.0	77.0	53.7
Srivastava index	86.9	67.7	73.0	83.6	77.3	54.5

BTT; β-thalassemia trait, Corr Dia; Correct diagnosis, HMA; Hypochromic microcytic anemia, IDA; Iron deficiency anemia, NPV; Negative predictive value, PPV; Positive predictive value, RBC; Red blood cell, RDW; RBC distribution width, Sen.; Sensitivity, Spe.; Specificity, YI; Youden's index

Table 4. *ROC analysis results and sensitivity and specificity levels according to the revised cut-off values*

Index	AUC	SE	Cut-off	Sen. (%)	Spe. (%)	P value
Ehsani index	0.868	0.021	9.3	72.1	87.6	<0.001
England and Fraser index	0.905	0.018	-4.7	80.1	87.6	<0.001
Green and King index	0.944	0.015	60.7	95.6	85.4	<0.001
Mentzer index	0.869	0.021	11.8	72.1	88.3	<0.001
RBC	0.127	0.021	5.39	21.3	19.0	<0.001
RDW	0.756	0.029	18.0	63.2	81.0	<0.001
RDW index	0.937	0.015	190.2	94.9	82.5	<0.001
Ricerca index	0.897	0.019	3.1	90.4	74.5	<0.001
Serdar index	0.936	0.015	0.66	92.6	84.7	<0.001
Shine and Lal index	0.742	0.031	773.5	61.0	82.5	<0.001
Sirdah index	0.904	0.018	21.5	94.1	72.3	<0.001
Srivastava index	0.822	0.026	3.7	71.3	83.2	<0.001

AUC; Area under the curve, RBC; Red blood cell, RDW; Red cell distribution width, SE; Standard error, Sen.; Sensitivity, Spe.; Specificity

Green and King Index and RDW Index not to be valid in differentiating BTT from IDA.[19,22,40] In all these studies, the YI for both the Green and King Index and the RDW Index has been reported to be in the unacceptable range (<50%), and some other erythrocyte indices such as the England and Fraser Index have been found to be more reliable. The controversial findings in different studies may be explained by the inclusion of cases with different age ranges, by accepting different cut-off values for some variables such as serum ferritin in defining IDA, and by other differences in study designs. In addition, the IDA and BTT characteristics may change from one population to another. For example, the percentage of severely affected IDA patients may be higher in populations where nutritional anemia is more prevalent, and these cases may have more pronounced abnormalities in erythrocyte indices. On the other hand, the erythrocyte phenotype of individuals with BTT may be affected by the underlying β-globin mutations and, if present, by the type of the accompanying α-globin mutations which vary from one population to another.

In the present study, 273 children with HMA could be included either into the IDA (n=136) or the BTT group (n=137). This moderate sample size and the almost equal number of cases in the two groups are among the strengths of this study. However, our study has some limitations, mainly due to its retrospective nature. First, the most reliable indicator of IDA is an increase in reticulocyte count and Hb level following sufficient oral iron therapy; however, we could not evaluate data regarding the response to iron therapy in our patients included in the IDA group. Second, although iron parameters such as serum ferritin levels were analyzed in most subjects in the BTT group, HbA_2 was available in only 37 (27.2%) of 136 cases in the IDA group (**Table 2**). This may be because physicians examining these patients did not suspect an underlying thalassemia trait because of a reason such as the resolution of HMA in the follow-up. Finally, we did not have any data about the presence or absence of any accompanying α-globin mutations, the presence of which may affect erythrocyte indices. However, we are of the opinion that α-globin mutations may have been present in only a few individuals among our cases, which may have had no significant effect on our results.

In conclusion, none of the erythrocyte indices are 100% specific or sensitive in differentiating BTT from IDA. Hb electrophoresis, and in selected cases, genetic analysis will offer a definitive diagnosis. However, our results suggest that the Green and King Index and RDW Index are superior to other indices analyzed here and could guide physicians in the initial discrimination between

IDA and BTT among children in the Isparta province of Turkey.

Ethical Approval: Süleyman Demirel University Faculty of Medicine Clinical Research Ethics Committee approval (February 2021 and 72 session number) was obtained before starting the study.

Informed Consent: Retrospective study.

Author Contributions: Uzunoğlu E: Surgical and Medical Practices, Data Collection or Processing, Analysis or Interpretation, Literature Search, Writing.; Yılmaz Keskin E: Surgical and Medical Practices, Concept, Design, Literature Search, Writing.

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