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Research Article

Comparison of haematological indices in diagnosing iron deficiency anaemia and thalassaemia: a retrospective study in Turkey

Demir eksikliği anemisi ve talasemi tanısında hematolojik indekslerin karşılaştırılması: Türkiye'de retrospektif bir çalışma

Nuray Üremiş[1*], Ergül Belge Kurutaş[1], Medine Çitil[2]

[1]Department of Medical Biochemistry, Medical Faculty, Kahramanmaras Sutcu Imam University, Kahramanmaras, Türkiye

[2]Kahramanmaraş Public Health Laboratory, Thalassaemia Laboratory, Kahramanmaras, Türkiye

Abstract

Aim: Accurate clinical differentiation between Iron Deficiency Anemia (IDA) and beta-thalassemia (β-TM) is critical for disease management and the determination of effective treatment strategies. Accordingly, in this study, we aimed to comparatively evaluate the laboratory methods and hematologic indices used for the diagnostic differentiation between IDA and thalassemia. The study examined the differences between routine biochemistry analyses of IDA and thalassemia and the effectiveness of eight different indices used to diagnose these diseases.

Material and Methods: Routine hematological parameters of patients diagnosed with IDA and β-TM Minor were analyzed retrospectively. Menzier Index (MI), Green and King Index (G&K), England and Fraser Index (E&F), Red Blood Cell Distribution Width Index (RDWI), Ricarca Index (RI), Srivastava Index (S), Sirdah Index (SI), and Ehsani Index were calculated and their diagnostic efficiency was evaluated.

Results: When eight discrimination indices were evaluated, G&K and E&F indices were the most effective in differentiating IDA and β-TM. These indices showed high sensitivity and specificity rates with Youden index values of 81.41% and 80.24%, respectively. As a result of the comparative evaluation of other discriminatory indices, the diagnostic performance order for distinguishing between IDA and β-TM was determined to be G&K > E&F > SI > RDWI > RI > MI > E > S > RBC.

Conclusion: Using various hematologic indices increases the diagnostic accuracy in differentiating IDA and β-TM. In particular, G&K and E&F indices may facilitate accurate diagnosis and enable more effective clinical management processes.

Keywords: Iron deficiency anemia, beta-thalassemia, hematologic index, red blood cell indices, erythrocyte parameter

Correspondence Author*: Nuray Üremiş, Department of Medical Biochemistry, Medical Faculty, Kahramanmaras Sutcu Imam University, Kahramanmaras, Türkiye
E-mail: nuremis@ksu.edu.tr
Orcid: 0000-0002-3958-4352
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Öz

Amaç: Demir eksikliği anemisi (DEA) ve beta-talasemi (β-TM) arasındaki doğru klinik ayrım, hastalık yönetimi ve etkili tedavi stratejilerinin belirlenmesi için kritik öneme sahiptir. Bu doğrultuda, bu çalışmada DEA ve talasemi arasındaki tanısal ayrım için kullanılan laboratuvar yöntemlerini ve hematolojik indeksleri karşılaştırmalı olarak değerlendirmeyi amaçladık. Çalışmada, DEA ve talaseminin rutin biyokimya analizleri arasındaki farklar ve bu hastalıkların teşhisinde kullanılan sekiz farklı indeksin etkinliği incelenmiştir.

Gereç ve Yöntemler: DEA ve β-TM Minör tanısı alan hastaların rutin hematolojik parametreleri retrospektif olarak analiz edildi. Menzier İndeksi (MI), Green and King İndeksi (G&K), England and Fraser İndeksi (E&F), Kırmızı Kan Hücresi Dağılım Genişliği İndeksi (RDWI), Ricarca İndeksi (RI), Srivastava İndeksi (S), Sirdah İndeksi (SI) ve Ehsani İndeksi hesaplandı ve tanısal etkinlikleri değerlendirildi.

Bulgular: Sekiz ayrım indeksi değerlendirildiğinde, G&K ve E&F indeksleri IDA ve β-TM'yi ayırt etmede en etkili indekslerdi. Bu indeksler sırasıyla %81,41 ve %80,24 Youden indeks değerleri ile yüksek duyarlılık ve özgüllük oranları gösterdi. Diğer ayırıcı indeksin karşılaştırmalı değerlendirmesi sonucunda, IDA ile β-TM'nin ayırt edilmesinde tanısal performans sırası G&K > E&F > SI > RDWI > RI > MI > E > S > RBC olarak saptanmıştır.

Sonuç: Çeşitli hematolojik indekslerin kullanılması DEA ve β-TM ayrımında tanısal doğruluğu artırmaktadır. Özellikle G&K ve E&F indeksleri doğru tanıyı kolaylaştırabilir ve daha etkili klinik yönetim süreçlerine olanak sağlayabilir.

Anahtar Kelimeler: Demir eksikliği anemisi, beta-talasemi, hematolojik indeks, kırmızı kan hücresi indeksleri, eritrosit parametreleri

Introduction

Thalassemia is a hemoglobinopathy of genetic origin characterized by defective globulin chains. It is estimated that approximately 1-5% of the population worldwide [1,2] and approximately 2-7% of the population in Asian and Middle Eastern countries carry thalassemia gene mutations [3,4]. Due to the high prevalence of genetic mutations, the demographic distribution of thalassemia, mainly concentrated in Asia, Mediterranean countries, Africa, and the Middle East, also varies [5]. The highest global prevalence was observed in children under 5 years of age and predominantly in the male sex [5]. Therefore, it is important to characterize the different phenotypic forms of thalassemia, which vary in age, gender, and geographical distribution, using global standard laboratory diagnostic methods [6].

Iron deficiency anemia (IDA) is a hematological disorder that causes anemia like thalassemia but with different pathophysiological mechanisms and biochemical characteristics [7]. Both disorders cause hypochromic, microcytic anemia and reduced oxygen-carrying capacity of red blood cells [8,9]. However, in IDA, the inability to synthesize hemoglobin is due to iron deficiency, whereas in thalassemia, defects in globin chains leading to structural defects are caused by genetic mutations [7,10]. Since iron deficiency anemia and thalassemia exhibit similar clinical symptoms, differential diagnostic tests between beta-thalassemia carriers and iron deficiency anemia are essential for disease management [11]. For this purpose, various indices calculated by routine tests such as Mentzer Index (MI), Green and King Index (G&K), England and Fraser Index (E&F), Red Cell Distribution Width Index (RDWI), Srivastava Index (S), Ricerca Index (R), Sirdah Index (SI) and Ehsani Index (E) are used [6,11]. These indices based on hematologic parameters are helpful for the diagnostic differentiation of IDA and β-TM.

Hematologic tests are among the primary methods for diagnosing IDA and thalassemia subtypes with autosomal recessive inheritance. Hematologic tests examining red blood cells' indices and structural properties, iron level determinations, hemoglobin electrophoresis, and microscopic evaluation of erythrocytes are widely used to differentiate IDA and β-TM [12]. In addition, various hematologic indices are also used to support clinical differentiation between

IDA and thalassemia. Accordingly, in our study, we aimed to comparatively evaluate the laboratory methods and hematologic indices used in determining the diagnostic differences between IDA and thalassemia. Our study examined the differences in routine biochemistry analyses of β-TM and IDA, and the efficacy of various hematologic indices used to diagnose these diseases was evaluated.

Material and Methods

Study design and data source

This retrospective cross-sectional study evaluates the differential hematological parameters of beta thalassemia minor and iron deficiency anemia. The study was accessed from the file records of minor cases of beta thalassemia detected within the scope of premarital screening tests performed at Kahramanmaraş Community Health Center and patients diagnosed with iron deficiency anemia. This study included 102 patients diagnosed as β-thalassemia carriers and 75 patients diagnosed with IDA who applied to Kahramanmaraş Community Health Center.

Venous blood samples used in the study were collected from participants who applied to Kahramanmaraş Community Health Centre under fasting conditions from the antecubital vein. After collection, the samples were transferred to tubes containing EDTA and serum separator tubes containing gel for biochemical analyses. Complete blood count analyses were performed on the same day, and serum samples for biochemical parameters were separated by centrifugation and stored at -80°C until analysis.

A minor with beta-thalassemia was diagnosed based on complete blood count, hemoglobin variant analysis, and related diagnostic criteria among individuals who applied for premarital testing. The diagnosis of iron deficiency anemia was based on the analysis of laboratory parameters such as low hemoglobin, serum ferritin, serum iron levels, and elevated total iron binding capacity, along with the clinical evaluation of the patients.

Inclusion and exclusion criteria

The iron deficiency anemia group included individuals with MCV below 70 fL, MCH below 27 pg, Hb below 12 g/dL, ferritin below 12 ng/mL, and total iron binding capacity above 400 µg/dL. In the thalassemia carrier group, individuals with HbA2 levels above 3.7%, MCV levels below 70 fL and MCH levels below 27 pg, ferritin levels above 12 ng/mL, and total iron binding capacities between 250-400 µg/dL were included.

Individuals with other genetic blood disorders, patients who have received blood transfusions in the last three months, patients who have undergone surgery in the previous six months, patients with acute or chronic diseases, and patients with hepatic or renal impairment were excluded from the study. In addition, women who are pregnant or breastfeeding, individuals who have used iron, folic acid, or vitamin B12 supplements in the last three months, patients with bone marrow disease, and patients diagnosed with hypothyroidism or hyperthyroidism were excluded due to possible effects on iron metabolism and hematological parameters.

Blood analyses and indices

In the study, ferritin levels were determined using Cobas e 602 auto analyzers (Roche Diagnostics, Germany) working with the electrochemiluminescence immunoassay (ECLIA) method, and complete blood count parameters (RBC, Hb, MCV, RDW, and MCH) were analyzed with Sysmex XN 3000 hematology analyzer (Sysmex, Kobe, Japan). Hemoglobin variant analysis was performed using the Bio-Rad Variant II Hemoglobin Testing System (Bio-Rad Laboratories, USA) using high-performance liquid chromatography (HPLC). Daily internal quality control (IQC) and external quality control (EQC) procedures were performed to ensure the reliability and accuracy of the analysis methods used in the study. The Cobas e 602 auto analyzers used for ferritin measurement were regularly calibrated using the calibration protocols provided by the manufacturer. The measurement range of the method was X-Y ng/mL and the inter- and intra-assay coefficients of variation were determined as A% and B%, respectively. In the Sysmex XN 3000 hematology analyzer used in complete blood count analyses, the accuracy and measurement reliability of the device were ensured by automatic quality control systems before each measurement.

In line with the data obtained from hematological parameters, eight different hematological indices were calculated using formulas defined in the literatüre: MI, G&K, RDWI, E&F, RI, S, SI ve E [11,13-16]. The mathematical formulae, clinical usage areas, and diagnostic cut-off values of these indices are presented in detail in Table 1. The study was approved by Kahramanmaraş Sütçü İmam University Medical Research Ethics Committee with protocol number 2025/05-388.

Tablo 1. Mathematical formulas that contribute to the differentiation between thalassemia minor and IDA.

Index	Formula	Use of Indexes	IDA	BTM	REF
MI	MCV/RBC MCV (femtolitre, fL) RBC (million/µL)	MI is used to differentiate microcytic anemias (anemias with low MCV). In IDA, MI > 13 is found because MCV decreases while RBC usually decreases. In BTM, although MCV is low, MI < 13 is found because the RBC count is high.	>13	<13	[29]
G&K	$MCV^2xRDW/Hbx100$ MCV (femtolitre, fL) RDW (%) Hb (g/dL)	G&K includes a combination of hematological parameters. In IDA, RDW is usually increased, and the G&K Index is usually > 72 with low Hb. RDW is usually normal or slightly increased in BTM. Therefore, the G&K Index is usually <72.	>72	<72	22]
RDWI	MCVxRDW/RBC MCV (femtolitre, fL) RDW (%) RBC (million/µL)	RDWI correlates the size heterogeneity of red blood cells with their numerical characteristics by combining MCV and RDW values. Since the size of erythrocytes is heterogenized in IDA, RDW is markedly increased. Therefore, the RDWI is usually > 220. In BTM, RDW was normal or slightly increased and, RBC count was usually elevated.	>220	<220	[30]
E&F	MCV−(5xHb)−RBC−3.4 MCV (femtolitre, fL) RDW (%) Hb (g/dL)	E&F differentiates microcytic anemias by combining erythrocyte volume and hemoglobin values. It is one of the hematological indices used to differentiate of IDA and BTM. MCV is significantly lower in BTM, but the decrease in MCV is less marked in IDA than in BTM.	> 0	≤ 0	[31]
SI	(MCV-RBC)-3xHb MCV (femtolitre, fL) RBC (million/µL) Hb (g/dL)	SI supports distinguishing between DEA and BTM using MCW RBC and HB values. MCV is significantly lower in BTM, but the decrease in MCV is less marked in IDA than in BTM.	> 27	< 27	[11]
RI	RDW/RBC RDW (%) RBC (million/µL)	The RI calculates the ratio of erythrocyte distribution width to red blood cell count, thus revealing differences in microcytic anemias. Since RDW is high and RBC is low in IDA, the index > 3.3. In BTM, since RDW is low and RBC is high, the index ≤ 3.3	> 3.3	<3.3	[15]
S	MCH/RBC MCH (pg) RBC (million/µL)	It is a hematological index differentiating IDA and BTM. In IDA, the index value is above 3.8 because RBC is low or normal, while in BTM, the index value remains below 3.8 because RBC is high.	>3.8	<3.8	[24]
E	MCV-(10xRBC) MCV (femtolitre, fL) RBC (million/µL)	It is a hematological index differentiating IDA and BTM. In IDA, the Ehsani Index is usually calculated above 15 due to low MCV and RBC values, whereas in BTM, the value of this index remains below 15 due to the high RBC count.	>15	<15	[11]
RBC	RBC RBC(million/µL)	RBC is a basic hematological parameter that measures the total number of blood cells. It is used to differentiate between different anemic conditions. In IDA, the RBC count may usually be below the limit of 5 million cells/microlitre, whereas in BTM, this value may be higher.	<5	>5	[16]

Abbrev.: RBC: Red Blood Cell Count, Hb: Blood hemoglobin level, MCV: Mean corpuscular volume, MCH: Mean Corpuscular Hemoglobin, RDW: Red Cell Distribution Width, MI: Mentzer Index, G&K: Green and King Index, RDWI: Red Blood Cell Distribution Width Index, E&F: England and Fraser Index, SI: Sirdah Index, RI: Ricerca Index, S: Srivastava Index, E: Ehsani Index

Statistical Analysis

Sensitivity, specificity, and Youden index values were calculated for each index. Sensitivity was expressed as the ratio of true positives to total positives, and specificity as the ratio of true negatives to total negatives. The youden index was calculated by subtracting 100 from the sum of sensitivity and specificity. Shapiro-Wilk tests were performed to evaluate the normality of the data. Group comparisons were made using the independent sample t-test for normally distributed data. Mann-Whitney U test was preferred as a non-parametric analysis method for non-normally distributed data. In order to apply parametric tests, the homogeneity of variance of the data was also checked using Levene's test. Statistical analyses were performed using GraphPad

Prism 9 software, and results with a p-value < 0.05 were considered statistically significant. Means and standard deviations of the data are shown as *p < 0.05, **p < 0.01 or ***p < 0.001.

Results

In our study, hematologic parameter and derived index analyses performed in patients diagnosed with β-TM and IDA revealed significant differences between the two groups. The data in Table 2 showed that RBC, Hb, and HCT counts were significantly higher in the β-TM group compared to the IDA group, while MCV and MCH values were lower in β-TM. In addition, Mentzer, G&K, E&F, and other hematologic indices were significantly lower in the β-TM group, demonstrating the potential of this parameter in differential diagnosis.

When the sensitivity, specificity, and Youden index values presented in Table 3 were analyzed, the E&F and G&K indices showed high performance in differentiating IDA and β-TM. The E&F index provided a Youden index of 80.24 with 92% sensitivity and 88% specificity in the IDA group, while the G&K index provided 85% sensitivity and 96% specificity, respectively, yielding a Youden index of 81.41. These findings reveal that E&F and G&K indices have superior discriminative power. In our study, the ranking of the discriminative performance of hematologic indices according to Youden index analysis was determined as G&K>E&F>SI>RDWI>RI>MI>E>S>RBC.

In Table 4, the evaluation of the number of patients correctly diagnosed for each index showed that the E&F and G&K indices provided the highest diagnostic accuracy, with 92% correct diagnosis. The Sirdah index stood out as a valuable alternative in clinical practice with an accuracy rate of 90%. In contrast, the Mentzer, Ehsani, and RBC count indices were 84%, and the Srivastava index was 83% accurate.

The data obtained show that E&F and G&K indices are reliable and preferable tools in clinical practice due to their high sensitivity, specificity, and diagnostic accuracy in differentiating β-TM and IDA. It also shows that the other indices evaluated can make important contributions to the differential diagnosis of microcytic anemias, effective use of hematologic indices, and accurate diagnosis.

Table 2. Hematological data and hematological indices of the study groups.

	BTM (n: 102)		IDA (n: 75)		
	Range	Mean ± SD	Range	Mean ± SD	P Value
Age	17-35	25.9± 4.81	20-51	36.2± 8.81	<0.001
Gender (male/female)	51/51		36/39		
RBC (×106/µL)	4.79-7.24	6.49 ± 3.30	3.88-5.54	4.82 ± 0.49	<0.001
Hb (g/dL) total	10.2-14.6	12.24 ± 1.42	8-11.6	10.06 ± 0.92	<0.001
HCT %	30.8- 48.6	38.18 ± 4.11	25.3-36.7	32.72 ± 2.30	<0.001
MCV (fL)	54.6-78.6	61.99 ± 6.29	57.2-78.6	68.18 ± 4.65	<0.001
MCH (pg)	16.9-27.3	19.89 ± 2.50	15.8-24.1	20.91 ± 1.97	<0.001
RDW (%)	14-20	17.27 ± 1.25	14.9-26.6	18.10 ± 2.15	<0.001
MI	7.55-15.53	10.11 ± 2.02	9.04-19.08	14.30 ± 1.90	<0.001
G&K	38.57-80.01	54.45 ± 8.13	45.53-168.32	84.64 ± 17.65	<0.001
E&F	-19.91-2.42	-9.13 ± 7.85	0.28-22.9	9.63 ± 5.94	<0.001
RDWI	129.82-259.53	173.44 ± 29.74	192.32-333.58	259.0 ± 49.25	<0.001
RI	2.47-4.38	2.79 ± 0.40	2.64-6.65	3.79 ± 0.58	<0.001
SI	9.93-28.65	18.75 ± 6.90	24.77-44.74	33.16 ± 5.03	<0.001
S	2.52-5.22	3.25 ± 0.71	2.79-5.75	4.39 ± 0.67	<0.001
E	-21.7-28.9	-2.96 ± 34.62	0.4-37	19.24 ± 7.64	<0.001

Abbrev.: RBC: Red Blood Cell Count, Hb: Blood hemoglobin level, MCV: Mean corpuscular volume, MCH: Mean Corpuscular Hemoglobin, RDW: Red Cell Distribution Width, MI: Mentzer Index, G&K: Green and King Index, E&F: England and Fraser Index, SI: Sirdah Index, RI: Ricerca Index, S: Srivastava Index, E: Ehsani Index

The statistical difference between the IDA and BTM groups was analyzed using an independent sample t-test. Data were presented as mean ± standard deviation (Mean ± SD), and p<0.05 was considered statistically significant.

Table 3. Sensitivity, specificity values, and Youden index to discriminate between β-TT and IDA in 177 patients.

Indices	Sensitivity (%)	Specificity (%)	Youden's index
Mentzer			
IDA	76	90	66.20
β-TM	90	76	
Green and King			
IDA	85	96	81.41
β-TM	96	85	
RDWI			
IDA	82	93	75.80
β-TM	93	82	
England and Fraser			
IDA	92	88	80.24
β-TM	88	92	
Sirdah			
IDA	88	91	79.18
β-TM	91	88	
Ricerca			
IDA	81	94	75.45
β-TM	94	81	
Srivastava			
IDA	81	84	65.65
β-TM	84	81	
Ehsani			
IDA	75	91	65.84
β-TM	91	75	
RBC count			
IDA	68	95	62.67
β-TM	95	68	

IDA: Iron Deficiency Anemia, β-TM: beta-thalassemia Sensitivity = true positive/(true positive + false negative); specificity = true negative/(true negative + false positive); Youden's index = (sensitivity + specificity) – 100.

Table 4. Number of IDA and β-TM patients correctly identified from each hematological index.

Indices (cut-offs)	β-TM (n=102)	IDA (n=75)	Total number of correctly diagnosed patients	Correctly diagnosed (%)
Mentzer				
IDA >13	92	18	149 (92 + 57)	84
β-TM <13	10	57		
Green and King				
IDA >65	98	11	162 (98 + 64)	92
β-TM <65	4	64		
RDWI				
IDA >220	95	13	157 (95 + 62)	89
β-TM <220	7	62		
England and Fraser				
IDA >0	90	3	163 (90 + 73)	92
β-TM <0	13	73		
Sirdah				
IDA >27	93	9	159 (93 + 66)	90
β-TM <27	9	66		
Ricerca				
IDA >3.3	96	14	157 (96 + 61)	89
β-TM <3.3	6	61		
Srivastava				
IDA >3.8	86	14	147 (86 + 61)	83
β-TM < 3.8	16	61		
Ehsani				
IDA >15	93	19	149 (93 + 56)	84
β-TM <15	9	56		
RBC count (×106/L)				
IDA <5	97	24	148 (97 + 51)	84
β-TM >5	5	51		

Discussion

The accurate diagnosis and clinical management of patients with microcytic anemia are of critical importance in the field of hematology [11]. Identifying hemoglobin variants has become an increasing clinical necessity in the premarital, prenatal, and postnatal periods [13]. The identification of relevant variants contributes to the prevention of severe and fatal subtypes of thalassemia syndromes, as well as the development of effective treatment strategies for IDA and thalassemia patients [14,15]. The performance of hematologic analyses and the identification of hemoglobin variants contribute significantly to the correct interpretation of the clinical diagnosis; however, current methods have limitations for the precise differentiation of IDA and β-TM patients [15-17]. Although HbA2 analysis is considered the gold standard for identifying β-thalassemia carriers, it alone is insufficient for screening mild cases of microcytic hypochromic anemia and preventing false positive results [18]. Therefore, discriminative indices based on formulations derived from hematologic parameters have been developed for the differential diagnosis of IDA and β-TM patients [17].

Many studies have demonstrated the superiority of these indices in differential diagnostic power, their clinical applicability, and their differences. However, several studies have reported that the differential diagnostic performance of these indices varies significantly [11,16,18-20]. The indices evaluated exhibited accuracy rates of approximately 60% to 90% in distinguishing whether hypochromic and microcytic anemia was due to β-TM or IDA. No discriminative

index developed so far has achieved 100% performance in sensitivity, specificity, and Youden index [17,21]. However, the comparative effectiveness of certain indices has been ranked according to their Youden index values [15,16,18-20]. Demir et al. defined the three most reliable indices as S&L, G&K, and E&F, respectively, according to the Youden index calculated by including pediatric patients with IDA and β-TM with Hb values between 8.5-11 g/dl [19]. On the other hand, Okan et al. determined the most reliable indices for the differentiation between IDA and β-TM as S&L > G&K > E&F = RDWI in their study, including IDA patients with Hb values below 8.5 g/dl [16]. Uzuncan et al. evaluated 12 different indices based on the hematologic data of the patients. They reported the Youden index of 69.8% and the 11T index with an accuracy of 81.2% as the most effective indices in the discrimination of IDA and β-TM [11]. Sırdah and colleagues reported that the Green-King formula and RDWI provided the highest reliability in distinguishing β-TM from IDA by evaluating 1272 β-thalassemia minor and 924 iron deficiency anemia patients recruited from the Palestinian population [20]. Villarroel et al. examined the variability of red blood cell indices in a total of 182 patients (51 β-TM and 131 IDA) in Chile; according to their results, G&K (0.923), Wongprachum (0.908), and Keikhaei (0.896) indices had the highest discriminatory power according to Youden index values [22]. In a study conducted by Matos and colleagues on a Brazilian population (β-TM = 47, IDA = 289), G&K and RDWI indices showed superior performance in discriminating IDA from β-TM compared to other hematologic indices [23]. Rastogi et al. reported that Sehgal index, Mentzer, and G&K were highly sensitive and specific in screening mild cases of microcytic hypochromic anemia and differentiating β-TM from IDA [18]. Vehapoğlu et al. reported that Mentzer, Ehsani, and Sirdah indices had high predictive value compared to Youden index results in a study conducted on 290 children with microcytic anemia with IDA and β-TM [15]. Tari et al. evaluated 120 IDA and 80 β-TM patients with hypochromic microcytic erythrocyte (RBC) morphology and reported the three most reliable formulation-based RBC indices as G&K, MI, and E&F, respectively [24]. In our study, 8 different indices were evaluated in terms of accuracy, specificity, and Youden index, and the number of IDA and β-TM patients correctly identified according to the indices was given. The Youden index percentages of the discriminant indices were determined as G&K 81.41%, E&F 80.24%, SI 79.18%, RDWI 75.80%, RI 75.45%, MI 66.20%, E 65.84%, S 65.65%, RBC 62.67%. The total percentage of correctly diagnosed patients was G&C 92%, E&F 92%, SI 90%, RDWI=RI 89%, MI=E=RBC 84%, S 83%. High sensitivity, specificity, Youden index, high true positive rate, and ease of calculation are expected in an ideal discrimination index [25,26]. In line with these criteria, the three indices with our study's highest predictive value were G&C, E&F, and SI, respectively. In the literature, findings have been reported in which indices with different formulations were applied to differentiate between β-TM and IDA using complete blood count data. However, a universal equation for mathematical indices developed based on populations with different genetic variations, age groups, patient numbers, and laboratory results could not be determined [15,20,23,27]. This has led to significant variability among the proposed discriminant indices based on high sensitivity, specificity, and Youden index. In this context, many discriminant index studies have encouraged researchers to work on finding the optimal equation, determining the most appropriate cut-off values, and developing new indices [22,28].

In conclusion, in addition to assessing HbA2 and iron status in patients with hypochromic microcytic anemia, calculating discriminant indices facilitates the differentiation of β-TM from IDA. As a result of the comparative evaluation of eight discriminatory indices, the diagnostic performance order for distinguishing IDA from β-TM was determined as G&K > E&F > SI > RDWI > RI > MI > E > S > RBC. In terms of the Youden index, the G&K (81.41%) and E&F (80.24%) indices stood out with the highest sensitivity and specificity values; this finding suggests that these indices may be particularly useful in clinical practice for distinguishing between IDA and β-TM. Therefore, large-scale sample studies of health centers' populations will allow for improved diagnostic accuracy and effectiveness of clinical management strategies.

Declaration of conflicting interests

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Ethics approval

The study was approved by Kahramanmaraş Sütçü İmam University Medical Research Ethics Committee with protocol number 2025/05-388.

Author Contributions

NÜ analysed the data and wrote the manuscript; EBK conceived the study and designed the experiments; MÇ collected and interpreted the data.

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