Better differential diagnosis of iron deficiency anemia from beta-thalassemia trait

Demir eksikliği anemisinin beta-talasemi taşıyıcılığından ayırıcı tanısının daha iyi yapılması

Fakher Rahim[1], Bijan Keikhaei[2]

[1]Research Center of Thalassemia & Hemoglobinopathies, Ahwaz Jondishapour University of Medical Sciences and Apadana Clinical Research Center, Apadana Private Hospital, Ahwaz, Iran
[2]Physiology Research Center, Ahwaz Jondishapour University of Medical Sciences, Ahwaz, Iran

Abstract

Objective: Iron deficiency anemia (IDA) and beta-thalassemia trait (ß-TT) are the most common forms of microcytic anemia. This study was conducted to compare the validity of various discrimination indices in differentiating β-TT from IDA by calculating their sensitivity, specificity and Youden's index.
Methods: Totally 323 subjects (173 children and 150 adults) with microcytic anemia were involved in this study. We calculated 10 discrimination indices in all patients with IDA and β-TT. We divided the patients into two different groups as younger or older than 10 years.
Results: None of the indices showed sensitivity and specificity of 100% in the patients older than 10 years, and in the patients younger than 10 years, only Shine & Lal index showed sensitivity close to 90% and specificity of 100%. The most accurate discriminative index for patients younger than 10 years was Shine & Lal and for those older than 10 years it was RDW index. According to Youden's index, Shine & Lal and RBC count showed the greatest diagnostic value in patients younger than 10 years and RDW and RBC count indices in those older than 10 years.
Conclusion: None of the indices was completely sensitive and specific in differentiation between β-TT and IDA. Mean and median mean cell Hb density (MCHD) were very close to normal values in both IDA and β-TT patients, but in the case of mean density of Hb/liter (MDHL), we found that the mean and median were significantly higher than normal values in β-TT and lower than normal values in IDA patients. In our study, Youden's index of RBC and Shine & Lal were the highest and most reliable indices in differentiating β-TT from IDA in the patients younger than 10 years. For patients older than 10 years, the most reliable discrimination indices were RBC and RDW. *(Turk J Hematol 2009; 26: 138-45)*
Key words: ß-Thalassemia trait, iron deficiency anemia, red blood cell, indices, discriminative indices

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Özet

Amaç: Demir eksikliği anemisi (DEA) ve beta-talasemi taşıyıcılığı, mikrositik aneminin en sık nedenleridir. Bu çalışma, β-talasemi taşıyıcılığının (β-TT) DEA'dan ayrımında kullanılan ayırt edici indekslerin duyarlılıklarını, özgüllüklerini ve Youden

Address for Correspondence: Dr. Fakher Rahim, Golsetan 1366, Ahwaz Jondishapour University of Medical Sciences, Ahwaz,Iran
Phone: 00986113362411 E-mail: fakherraheem@yahoo.com

indekslerini hesaplayarak, ayırıcı tanıdaki önemlerini karşılaştırmak amacıyla yürütülmüştür.
Materyal ve Metod: Mikrositik anemili toplam 323 kişi (173 çocuk ve 150 erişkin) bu çalışmaya dahil edilmiştir. DEA ve β-TT'li tüm hastalarda 10 ayırt edici indeks değerlendirilmiştir. Hastalar 10 yaşından küçük ve 10 yaşından büyük olmak üzere iki farklı gruba ayrılmıştır.
Bulgular: İndekslerden hiçbiri 11 yaşından büyük hastalarda %100 duyarlılık ve özgüllük göstermemiş, sadece Shine ve Lal indeksleri 10 yaşından küçük hastalarda %90'a yakın bir duyarlılık ve %100 özgüllük göstermiştir. En hassas ayırt edici indeks olarak 10 yaşından küçük hastalar için Shine ve Lal indeksleri, 11 yaşından büyük hastalar için ise RDWI indeksi bulunmuştur. Shine ve Lal ve kırmızı kan hücresi sayısı için Youden indeksinin ve RDWI ve kırmızı kan hücresi sayısı için Youden indeksinin, sırasıyla 10 yaşından küçük ve 10 yaşından büyük hastalar için en yüksek tanısal değere sahip olduğu görülmüştür.
Sonuç ve Tartışma: İndekslerden hiçbiri β-TT ve DEA ayrımında tam olarak duyarlı ve özgül değildi. Hem DEA hem de β-TT hastaları için ortalama ve ortanca MCHD değerleri normale çok yakındı, fakat MHDL durumunda ortalama ve ortancaların, β-TT için normal değerlerden anlamlı olarak yüksek ve EDA hastalarının değerlerlerinden düşük olduğunu bulduk. Çalışmamızda, Shine ve Lal ve kırmızı kan hücresi sayısının Youden indeksi 10 yaşından küçük hastalarda β-TT ve DEA ayrımında en yüksek ve en güvenilir ayırt edici indeks, 11 yaşından büyük hastalar için ise kırmızı kan hücresi ve RDWI en güvenilir indeksler olarak bulunmuştur. *(Turk J Hematol 2009; 26: 138-45)*
Anahtar kelimeler: β-talasemi taşıyıcılığı, demir eksikliği anemisi, kırmızı kan hücresi, İndeksler, ayırıcı indeksler

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Introduction

The most commonly encountered disorders with mild microcytic anemia are iron deficiency anemia (IDA) and thalassemia trait (TT) [1,2]. The gene frequency of β-thalassemia, however, is high and varies considerably from area to area, having its highest rate of more than 10% around the Caspian Sea and Persian Gulf. α -thalassemia is very rare in Iran, and there is no clear report of the prevalence for the country as a whole. Results of genotyping for the two most common α-thalassemia single gene deletions ($-\alpha^{3.7}$ and $-\alpha^{4.2}$) showed that the $-\alpha^{3.7}$ deletion was a common cause of microcytic and hypochromic anemia in Iran [3]. The prevalence of the disorder in other areas is between 4-8%. In Isfahan, a city built around the river Zayandeh-Rood in the central part of Iran, the frequency rises to about 8%. In the Fars Province, in southern Iran, the gene frequency is also high and reaches 8-10% [4]. To date, many discrimination indices have been reported using red blood cell (RBC) indices obtained by automated blood count. Many authors have calculated the sensitivity and specificity of these indices in the distinction between IDA and TT [2]. They proposed that the diagnosis could be established without having to resort to the more time-consuming methods such as transferrin saturation (TS), ferritin and hemoglobin A_2 (HbA_2) levels. However, none of these indices showed a sensitivity and specificity of 100% in prediction of IDA and TT. Some showed considerable sensitivity for IDA or TT, but not specificity [5]. Youden's index provides an appropriate measure of validity of a particular technique or question by taking into account both sensitivity and specificity [6,7]. In this study, we compared the validity of various indices in differentiating β-TT from IDA by calculating their sensitivity, specificity and Youden's index. In some published articles, the validity of all of the defined discrimination indices were compared in the same patient groups, and it was shown that the RBC count is one of the two most accurate indices available [8-11].

Materials and Methods

Totally 323 subjects (173 children and 150 adults) with microcytic anemia were involved in this study. We divided the patients into two categories according to age as 1 to 10 years and 11 to 57 years. The division was done using a method based on difference in red cell indices such as mean corpuscular volume (MCV) published by Hermiston and Mentzer [12]. Venous blood was drawn with the usual precautions after the patient had been lying quietly for at least 20 minutes. The hematological analyses were carried out with a Coulter Counter (M530, Coulter Electronics Ltd., Luton, UK) particle counter. Hb concentration, RBC count, and total leukocyte count were recorded, and liquid chromatography technique was used for determination of HbA_2 [13]. Serum iron (SI), serum iron binding capacity (SIBC), serum ferritin, and HbA_2 values were determined in all. Diagnosis of IDA was based on a Hb level of <11.4 and <12.9 g/dl and MCV of <81 and <84.8 fl in those younger or older than 10 years, respectively, and a ferritin value of <10 ng/ml and TS of <12% in both age groups. According to these criteria, 114 children and 56 adults of the 323 patients were diagnosed as IDA. The patients with Hb value <8.7 g/dl were excluded in order to not confuse more severe IDA with β-TT. In the remaining 59 patients younger than 10 years and 94 patients older than 10 years, the diagnosis of TT was based on presence of microcytosis and elevated levels of HbA_2 (>3.5%). RBC count and red blood cell distribution width (RDW) [14,15] were obtained with Coulter Counter and the other discrimination indices were calculated by using RBC indices as defined below:

Mentzer Index (MI): MCV/RBC [16],

Shine and Lal Index (S&L): MCV^2 × mean corpuscular hemoglobin (MCH) × 0.01[17],

England and Fraser (E&F): MCV - RBC - (5 × Hb) – k [18], k is calculated to be 4.54 in our counter as described in the published report [19],

Srivastava Index (SI): MCH/RBC [20],

Green and King Index (G&K): MCV^2 × RDW/100 × Hb [21],

RDW Index (RDWI): MCV × RDW/RBC [22],

Mean Cell Hb Density (MCHD) Index: MCH/MCV [23],

Mean Density of Hb/Liter of Blood (MDHL) Index: MCHD × RBC [23].

Differential value for each of above indices was applied as given in the original published reports [6-22].

We calculated two hematological parameters including MDHL and MCHD in healthy male and female subjects and patients separately using data from Shafa Hospital based on the method described by Telmissani et al. [23]. The diagnostic method was that patients with values equal to or below the mean of healthy subjects were most likely to have IDA, and patients with values higher than the mean of healthy subjects were most likely to have β-TT. We computed mean and median of MCHD and MDHL for those 323 patients and compared the obtained values to the normal values, which are shown in Table 6. We attempted to compare 10 different discrimination indices with respect to five parameters include sensitivity, specificity, positive predictive value, negative predictive value, and Youden's index.

Sensitivity and specificity were calculated according to standard formulas, namely: Sensitivity = (TP) / (TP + FN) and specificity = (TN) / (TN + FP), where TP = true positives, FN = false negatives, TN = true negatives and FP = false positives. The predictive values (PV), whether positive (+) or negative (–), were similarly calculated, with +PV being (TP) / (TP + FP) and –PV being (TN) / (TN + FN). Finally, Youden's index was calculated as follows: (Sensitivity + specificity) - 100.

Results

Of the 323 patients, 170 (114 children, 56 adults) were diagnosed to have IDA and 153 (59 children, 94 adults) were diagnosed to have β-TT. The hematological data of the groups are shown in Table 1. The different indices for patients younger or older than 10 years were calculated individually. The differential value for each index in differentiation between β-TT and IDA and the number and proportion of correctly identified patients (true positives) calculated using these indices are shown in Table 2 (those younger than 10 years) and Table 4 (those older than 10 years). The sensitivity, specificity, positive and negative predictive values and Youden's index of each index in differentiation between β-TT and IDA are shown in Table 3 (those younger than 10 years) and Table 5 (those older than 10 years). All of the indices showed overlapping in the patients with β-TT and IDA. The overlaps were between 12.5 and 21.1% in RDW and between 4.3 and 5.49 × 10^{12} in RBC for patients younger than 10 years, while these respective values were between 11.4 and 25.1% and between 4.38 and 5.94 × 10^{12} in those older than 10 years. None of the indices was completely sensitive or specific in differentiation between β-TT and IDA. Youden's indices in decreasing order in patients younger than 10 years were as follows: S&L > RBC > SI > MI > E&F > MDHL > G&K > RDWI > RDW > MCHL, and in patients older than 10 years were: RDWI > RBC > MI > E&F > SI > MDHL > RDW > S&L > G&K > MCHL. The scatter-gram of the hematological data of all patients showed significant differences between these two disorders (Figure 1).

However, MDHL, which showed that patients with values equal to or below the mean of healthy subjects (for younger than 10 years: 1.71 in males and 1.98 in females; for older than 10 years: 1.80 in males and 1.76 in females) were most likely to have IDA and those whose MDHL value was greater than the mean were more likely to have β-TT. We computed mean and median of MCHD and MDHL for these 323 patients and compared results with the normal values (Table 6). We found that mean and median MCHD values were very close to normal in both IDA and β-TT patients, while mean and median MDHL values were significantly more than normal values in β-TT and lower than normal in IDA patients (Table 7).

Discussion

The most frequently encountered diseases with mild microcytic anemia are TT and IDA. However, in addition to genetic counseling for identification of thalassemia carriers in order to prevent the birth of thalassemia patients, differentiating TT from IDA is warranted because the thalassemia heterozygote should not be given iron in a vain attempt to normalize MCV. The diagnosis of β-TT is established by the presence of characteristic RBC microcytosis and elevated levels of HbA_2 [24]. Decreased levels of SI, TS and ferritin with increased

Table 1. Hematological data of study groups

	IDA n=170				β - TT n=153			
	Younger than 10 years (n=114)		Older than 10 years (n=56)		Younger than 10 years (n=59)		Older than 10 years (n=94)	
Hematological data	Range	Mean [± SD]	Range	Mean [± SD]	Range	Mean [±SD]	Range	Mean [±SD]
Hb (g/dl)	8.7-11.4	10.12 (0.72)	8.7-12.9	10.25 (1.30)	9.1-12.25	10.45 (0.92)	8.7-15.4	11.6 (1.42)
RBC	3.7-5.49	4.45 (0.56)	3.57-5.94	4.48 (0.97)	4.3-6.95	5.66 (0.58)	4.38-7.71	5.94 (0.7)
MCV (n)	51-80.7	69.7 (7.36)	57.1-84.8	71.64 (8.56)	50-75	58.25 (5.26)	54-80	61.41 (5.21)
MCH (pg)	14.7-26.7	22.12 (3.31)	16-27.6	22.16 (3.47)	16.4-24	18.59 (1.82)	15-26	19.45 (2.00)
MCHC (g/dL)	22-35.1	31.14 (3.46)	24-40.4	30.23 (9.19)	27-39.4	32.6 (2.9)	24.1-47.3	36.12 (4.37)
RDW (%)	12.5-24.2	14.20 (4.21)	11.4-30.2	15.60 (6.11)	10.5-21.1	11.79 (6.75)	10.1-25.1	15.12 (2.37)
SI (µg/dl)	3.8-31	20.03 (8.01)	4.8-37	21.09 (8.42)	45-196	75.8 (29.1)	58-262	88.01 (33.6)
SIBC (µg/dl)	275-480	378.6 (70.01)	315-570	429.7(72.1)	224-425	321.7(36.8)	257-478	345.5(47.34)
Ferritin (ng/ml)	0.9-9.2	4.23(2.01)	1.2-9.9	4.57 (1.99)	12-85.4	31.09 (6.79)	12-92	37.05 (7.34)
TS (%)	0.68 -8.1	3.81 (2.10)	0.85-10.05	4.42 (2.28)	12.8-57	21.09(1.15)	15-67	26.84 (1.23)

β-TT: Beta thalassemia trait; Hb: Hemoglobin; IDA: Iron deficiency anemia; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; MCV: Mean corpuscular volume; RBC: Red blood cells; RDW: Red blood cell distribution width; SI: Serum iron; SIBC: Serum iron binding capacity; TS: transferrin saturation

Table 2. The differential values of each discrimination index and correctly identified number ofof patients in the group younger than 10 years

Differential values	IDA (n=114)	β - TT (n=59)	Total correctly identified patients (n=173)	Percentage of correctly identified patients (%)
E & F				
IDA > 0	105 †	17	147 (105+42)	85%
βTT < 0	9	†42		
SI				
IDA > 3.8	†105	11	153 (105+48)	89%
βTT < 3.8	9	†48		
RBC				
IDA < 5	†101	4	151 (101+50)	90%
βTT > 5	13	†55		
MI				
IDA > 13	†97	4	152 (97+55)	88%
βTT < 13	17	†55		
RDW				
IDA > 14	99	29	129 (99+30)	74%
βTT < 14	15	30		
G & K				
IDA > 65	†97	10	146 (97+49)	84%
βTT < 65	17	†49		
RDWI				
IDA > 220	†82	2	139 (82+57)	81%
βTT < 220	32	†57		
S & L				
IDA > 1530	†101	0	160 (101+59)	92%
βTT < 1530	13	†59		

†True positives; βTT: Beta thalassemia trait; IDA: Iron deficiency anemia; E & F: England and Fraser index; G & K: Green and King index; MI: Mentzer index; RBC: Red blood cells; RDW: Red blood cell distribution width; RDWI: Red blood cell distribution width index; SI: Srivastave index; S & L: Shine and Lal index

Table 3. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and Youden's index of each discrimination index in prediction of IDA and βTT groups younger than 10 years

Group	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Youden's Index
S & L					
IDA	89	100	100	82	89
βTT	100	89	82	100	
RBC					
IDA	89	93	96	81	82
βTT	93	89	81	96	
MI					
IDA	85	93	96	76	79
βTT	93	85	76	96	
SI					
IDA	92	81	91	84	73
βTT	81	92	84	91	
RDWI					
IDA	72	97	98	64	69
βTT	97	72	64	98	
G & K					
IDA	85	83	90	74	68
βTT	83	85	74	90	
E & F					
IDA	92	71	86	82	63
βTT	71	92	82	86	
MDHL					
IDA	80	72	85	66	54
βTT	72	80	66	85	
RDW					
IDA	87	51	77	67	38
βTT	51	87	67	77	
MCHD					
IDA	96	6	66	44	2
βTT	6	96	44	66	

βTT: Beta thalassemia trait; E & F: England and Fraser index; G & K: Green and King index; IDA: Iron deficiency anemia; MI: Mentzer index; RBC: Red blood cells; RDW, Red blood cell distribution width; RDWI: Red blood cell distribution width index; SI, Srivastave index; S & L: Shine and Lal index

Table 4. The differential values of each discrimination index and correctly identified number of patients in the group older than 11 years

Differential values	IDA (n=56)	β - TT (n=94)	Total correctly identified patients (n=150)	Percentage of correctly identified patients (%)
RDWI				
IDA > 220	51ψ	5	140 (51+89)	93%
βTT < 220	5	89ψ		
RBC				
IDA < 5	47ψ	6	135 (47+88)	90%
βTT > 5	9	88ψ		
MI				
IDA > 13	45ψ	5	134 (45+89)	89%
βTT < 13	11	89ψ		
E & F				
IDA > 0	52ψ	16	130 (52+78)	87%
βTT < 0	4	78ψ		
SI				
IDA > 3.8	47ψ	15	126 (47+79)	84%
βTT < 3.8	9	79ψ		
RDW				
IDA > 14	50	26	118 (50+68)	79%
βTT < 14	6	68		
S & L				
IDA > 1530	13ψ	1	106 (13+93)	70%
βTT < 1530	43	93ψ		
G & K				
IDA > 65	52ψ	45	101 (52+49)	67%
βTT < 65	4	49ψ		

ψ True positives; βTT: Beta thalassemia trait; IDA: Iron deficiency anemia; E & F: England and Fraser index; G & K: Green and King index; MI: Mentzer index; RBC: Red blood cells; RDW: Red blood cell distribution width; RDWI: Red blood cell distribution width index; SI: Srivastave index; S & L: Shine and Lal index

Table 5. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and Youden's index of each discrimination index in prediction of IDA and βTT groups in patients older than 11 years

Group	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Youden's Index
RDWI					
IDA	91	95	91	95	86
βTT	95	91	95	91	
RBC					
IDA	84	94	87	91	78
βTT	94	84	91	87	
E & F					
IDA	93	83	76	95	76
βTT	83	93	95	76	
MI					
IDA	80	95	90	89	75
βTT	95	80	89	90	
SI					
IDA	93	83	76	95	68
βTT	83	93	95	76	
MDHL					
IDA	79	87	79	87	66
βTT	87	79	87	79	
RDW					
IDA	89	72	66	92	62
βTT	72	89	92	66	
G & K					
IDA	93	52	54	92	45
βTT	52	93	92	54	
S & L					
IDA	23	99	93	68	22
βTT	99	23	68	93	
MCHD					
IDA	96	6	38	75	2
βTT	6	96	75	38	

βTT: Beta thalassemia trait; E & F: England and Fraser index; G & K: Green and King index; IDA: Iron deficiency anemia; MI: Mentzer index; RBC: Red blood cells; RDW, Red blood cell distribution width; RDWI: Red blood cell distribution width index; SI: Srivastave index; S & L: Shine and Lal index

levels of SIBC are the main diagnostic criteria for IDA [25]. Less time-consuming methods are based on the calculation of discrimination indices from RBC indices obtained during routine complete blood count. However, later studies have shown that the different reported indices correctly identify only 61–91% of the patients with microcytic anemia due to TT or IDA. As each index showed overlapping values in patients with TT and IDA, none of them was entirely satisfactory in discriminating between these conditions [26]. We calculated each discrimination index in the same patient groups with β-TT and IDA. In addition to calculating the sensitivity, specificity, positive predictive value and negative predictive value of each discrimination index, we also calculated Youden's index in their differentiation between β-TT and IDA.

In 2007, Suad et al. [10] in their research work applied and compared nine well-documented discriminant functions in a population of 153 confirmed cases of microcytic anemias and measured validity using Youden's index. They showed that the E&F index had the highest Youden's index value (98.2%) in correctly differentiating between IDA and α - and ß-thalassemia minor, while the S&L index was found ineffective in differentiating between microcytic anemias in their study. Finally, they concluded that the E&F index showed with great sensitivity and specificity to be the best discriminant function to differentiate between IDA and thalassemia minor cases. Our data showed that the S&L index had the highest Youden's index, with a value of 89%, in correctly differentiating between IDA and ß-thalassemia minor among patients aged younger than 10 years, while RDW and RBC count indices showed the highest Youden's index, with values of 93% and 90%, respectively, in the patients older than 10 years.

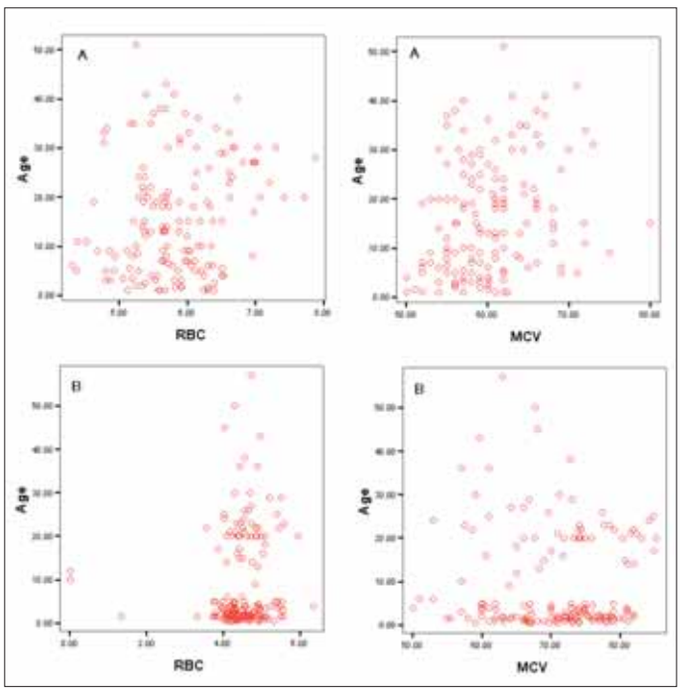

Figure 1. Scattergram of hematological data for β - thalassemia trait (A) and iron deficiency anemia (B) showed clear difference between these two disorders

In another research work, Ntaios et al. [11] examined the diagnostic accuracy of six discrimination indices in the differentiation between IDA and β-TT. He calculated these indices in 373 patients (205 men, 168 women) with β-TT and 120 patients (50 men, 70 women) with IDA, as well as their sensitivity, specificity, positive and negative prognostic value, efficiency, and Youden's index. The G&K index showed the highest reliability, followed by E&F, RBC count, MI, and RDWI. On the contrary, RDW completely failed to differentiate between IDA and β-TT. G&K proved to be the most reliable index, as it had the highest sensitivity (75.06%), efficiency (80.12%), and Youden's index (70.86%) for the detection of β-TT. In comparison, our data showed Youden's indices in decreasing order in patients younger than 10 years to be as follows: S&L > RBC > SI > MI > E&F > MDHL > G&K > RDWI > RDW > MCHL, and in patients older than 10 years as: RDWI > RBC > MI > E&F > SI > MDHL > RDW > S&L > G&K > MCHL. S&L, with the highest efficiency (92%), sensitivity (100%) and YI (89%) in the former group, and RDWI, with highest efficiency (93%), sensitivity (95%) and YI (86%) in latter group, proved to be the most reliable indices.

In recent research work published by Beyan et al. [9] in 2007, they attempted to evaluate the predictive value of these indices in the differential diagnosis of IDA and β-TT in adult cases. The study consisted of 45 IDA cases (36 women and 9 men, age range 17–57 years) and 66 β-TT cases (41 women and 25 men, age range 14–74 years). Patient groups were evaluated according to RBC, RDW, MI, S&L, E&F, SI, G&K, RDWI, and Ricerca index. Sensitivity, specificity, positive and negative predictive values, and Youden's index were calculated. They concluded that none of these formulations was superior to RBC value obtained from automated analyzers in adult cases with IDA and β-TT, and that total body iron status and HbA_2 level should be obtained for accurate differential diagnosis of IDA and β-TT until more efficient tools are developed. Our data showed that some of those indices could be used as efficient tools for differentiating between these two disorders.

Table 6. The normal values of Mean Cell Hemoglobin Density (MCHD) and Mean Density of Hemoglobin per Liter of blood (MDHL)

Variables	Male		Female	
	Group 1	Group 2	Group 1	Group 2
MCHD				
Median	0.32	0.32	0.32	0.31
Mean [± SD]	0.32 (0.01)	0.319 (0.02)	0.32 (0.01)	0.31 (0.01)
MDHL				
Median	1.71	1.98	1.8	1.76
Mean [± SD]	1.76 (0.22)	1.99 (0.28)	1.8 (0.19)	1.78 (0.17)

Group 1: Normal subjects who were younger than 10 years; Group 2: Normal subjects who were older than 10 years

Table 7. Mean Cell Hemoglobin Density (MCHD) and Mean Density of Hemoglobin per Liter of blood (MDHL) according to age groups and sex in IDA and βTT

	Patients younger than 10 years								Patients older than 10 year							
	Males				Females				Males				Females			
Differential values	IDA	βTT	Total correctly identified patients	Percentage of correctly identified patients (%)	IDA	βTT	Total correctly identified patients	Percentage of correctly identified patients (%)	IDA	βTT	Total correctly identified patients	Percentage of correctly identified patients (%)	IDA	βTT	Total correctly identified patients	Percentage of correctly identified patients (%)
MCHD																
IDA	38	27			72	28			4	41			50	47		
βTT	1	2	40 (38+2)	58%	4	2	74 (72+2)	69%	0	5	9 (5+4)	18%	2	1	51 (50+1)	51%
Total	39	29			76	30			4	46			52	48		
MDHL																
IDA	39	13			04	3			3	9			41	3		
βTT	0	16	55 (39+16)	80%	22	27	81 (54+27)	77%	1	37	40 (3+37)	80%	11	45	86 (41+46)	86%
Total	39	29			76	30			4	46			52	48		

Youden's index takes into account both sensitivity and specificity and gives an appropriate measure of validity of a particular question or technique. In our study, Youden's index of RBC count and RDWI were the highest and they were the most reliable discrimination indices in differentiating β-TT from IDA in patients older than 10 years. For patients younger than 10 years, the most reliable discrimination indices were RBC and S&L. These indices are not very important today given the availability of Hb electrophoresis. As physicians, how many of us memorize these formulas and use them in our daily practice in crowded outpatient settings? However, we do consider MCV and RBC and MI, which is easy to calculate. How many of us would be brave enough to not study Hb electrophoresis in a woman with mild hypochromic anemia unresponsive to iron therapy who was planning a pregnancy? Our calculated normal values of MCHD and MDHL were very close to those published by Telmissani et al. [23]. Although we found that mean and median values of MDHL in IDA were significantly lower than those in β-TT and normal values, Youden's index of MDHL was not found to be adequately high in either age group.

In conclusion, in patients with microcytic anemia, if β-TT or IDA is shown in a patient younger than 10 years with correct measures on S&L and RBC and in a patient older than 10 years with correct measures on RBC and RDW indices, the diagnoses are likely to be correct. However, in a small number of patients, it would still be necessary to study body iron status or HbA_2 for accurate diagnosis.

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References

1. Wharton BA. Iron deficiency in children: detection and prevention. Br J Haematol 1999;106:270–80.
2. Lukens JN. The thalassemias and related disorders: an overview. In: Lee GR, et al., editors. Wintrobe's Clinical Hematology. 10th ed. Giza: Mass Publishing, 1999: 405-33.
3. Haghshenas M, Zamani J. [Thalassemia]. 1st ed. Shiraz; Shiraz University of Medical Sciences Publishing Center, 1997. [Book in Persian]
4. Neishabury M, Oberkanins C, Moheb LA, Pourfathollah AA, Kahrizi K, Keyhany E, Krugluger W, Najmabadi H. High prevalence of the $-\alpha^{3.7}$ deletion among thalassemia patients in Iran. Hemoglobin 2003;27:53-5.
5. Lin CK, Lin JS, Chen SY, Jiang ML, Chia CF. Comparison of hemoglobin and red blood cell distribution width in the differential diagnosis of microcytic anemia. Arch Pathol Lab Med 1992;116:1030-2.
6. Pekkanen J, Pearce N. Defining asthma in epidemiological studies. Eur Respir J 1999;14:951-7.
7. Burney PG, Chinn S, Britton JR, Tattersfield AE, Papacosta AO. What symptoms predict the bronchial response to histamine? Evaluation in a community survey of the bronchial symptoms questionnaire (1984) of the International Union Against Tuberculosis and Lung Disease. Int J Epidemiol 1989;18:165–73.
8. Demir A, Yarali N, Fisgin T, Duru F, Kara A. Most reliable indices in differentiation between thalassemia trait and iron deficiency anemia. Pediatr Int 2002;44:612-6.

9. Beyan C, Kaptan K, Ifran A. Predictive value of discrimination indices in differential diagnosis of iron deficiency anemia and beta-thalassemia trait. Eur J Haematol 2007;78:524-6.
10. Suad MA, Anwar M Al-A, Doa'a A. Validity assessment of nine discriminant functions used for the differentiation between iron deficiency anemia and thalassemia minor. J Trop Pediatr 2007;53:93-7.
11. Ntaios G, Chatzinikolaou A, Saouli Z, Girtovitis F, Tsapanidou M, Kaiafa G, Kontoninas Z, Nikolaidou A, Savopoulos C, Pidonia I, Alexiou-Daniel S. Discrimination indices as screening tests for beta-thalassemic trait. Ann Hematol 2007;86:487-91.
12. Hermiston ML, Mentzer WC. A practical approach to the evaluation of the anemia child. Pediatr Clin North Am 2002;49:877-91.
13. Turpelnen U. Liquid-chromatographic determination of hemoglobinA2. Clin Chem 1986;3216:999-1002.
14. Klee GG, Fairbanks VF, Pierre RV, Virgh D, O'Sullivan MB. Routine erythrocyte measurements in diagnosis of iron-deficiency anemia and thalassemia minor. Am J Clin Pathol 1976;66:870-7.
15. Bessman JD, Feinstein DI. Quantitative anisocytosis as a discriminant between iron deficiencies and thalassemia minor. Blood 1979;53:288-93.
16. Mentzer WC. Differentiation of iron deficiency from thalassemia trait. Lancet 1973;1:882.
17. Shine I, Lal S. A strategy to detect beta-thalassemia minor. Lancet 1977;1:692-4.
18. England JM, Fraser PM. Differentiation of iron deficiency from thalassemia trait by routine blood-count. Lancet 1973;1:449-52.
19. England JM, Fraser P. Discrimination between iron-deficiency and heterozygous-thalassemia syndromes in differential diagnosis of microcytosis. Lancet 1979;1:145-8.
20. Srivastava PC, Bevington JM. Iron deficiency and-or thalassemia trait. Lancet 1973;1:832.
21. Green R, King R. A new red blood cell discriminant incor¬porating volume dispersion for differentiating iron deficiency anemia from thalassemia minor. Blood Cells 1989;15:481-95.
22. Jayabose S, Giavanelli J, Levendoglu-Tugal O, Sandoval C, Özkaynak F, Visintainer P. Differentiating iron deficiency anemia from thalassemia minor by using an RDW-based index. J Pediatr Hematol 1999;21:314.
23. Telmissani OA, Khalil S, George TR. Mean density of hemoglobin per liter of blood: a new hematologic parameter with an inherent discriminant function. Lab Haematol 1999;5:149-52.
24. Olivieri NF. The beta – thalassemias. N Engl J Med. 1999;341:99-109.
25. Oski FA. Iron deficiency in infancy and childhood. N Engl J Med 1993;329: 190-3.
26. Raper AB. Differentiation of iron-deficiency anaemia from thalassaemia trait. Lancet 1973;1:778.