Iron Deficiency Anemia and β-Thalassemia Minor Differantiation With Hematological Indices

Demir Eksikliği Anemisi ve Beta Talasemi Minör Ayırımında Kullanılan Hematolojik İndeksler

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ABSTRACT

Objective: Iron Deficiency Anemia (IDA) and Beta Thalassemia Minor (BTM) are the most common causes of microcytic hypochromic anemia. In the last four decades many formulas have been proposed to discriminate between two common causes of microcytic hypochromic anemia. An ideal discrimination index should have a high sensitivity and specificity and should be easy to calculate. The distinction between IDA and BTM is important in many respects. In our study, the performance of twelve indices were tested to distinguish IDA from BTM, two common causes of microcytic anemia.

Materials and methods: There were two groups which were evaluated as IDA and BTM, we examined the diagnostic accuracy of five red cell parameters [red blood cell (RBC), mean corpuscular volume (MCV), hemoglobin (Hb) ,mean corpuscular hemoglobin (MCH), red blood cell distribution width (RDW)] and we calculated twelve indices [Menzier Index (MI), Green and King (GK), Red Blood Cell Distribution Width Index (RDWI), England & Fraser (EF), RBC, Sirdah Index (SI), Ricarca Index (R), Srivastava (S), Shine & Lal (SL), Ehsani (E), Red Cell Distribution Width (RDW), 11T].

Results: Among the twelve discrimination indices, 11T index was the most efficient in differentiating IDA from BTM. 11T index had the highest Youden's index (69,8%) and accuracy (81,2%).

Conclusion: 11T index was the most effective in discriminating IDA from BTM. RBC, GK, SI and RDWI could be used as a screening tool rather than discrimination indices.

Key Words: Anemia; beta-thalassemia; Iron-Deficiency; erythrocyte indices

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ÖZET

Amaç: Demir Eksikliği Anemisi (IDA) ve Beta Talasemi Minör (BTM), mikrositer hipokrom aneminin en sık nedenlerindendir. Son yıllarda, mikrositer hipokrom aneminin bu nedenleri arasında ayrımın yapılması için birçok formül önerilmiştir. İdeal bir ayrım indeksi yüksek duyarlılığa ve özgüllüğe sahip olmalı ve hesaplanması kolay olmalıdır. IDA ve BTM arasındaki ayrım pek çok açıdan önemlidir. Bizim çalışmamızda 12 indeksin performansı, mikrositik aneminin iki yaygın nedeni olan BTM'yi IDA'dan ayırt etmek için test edildi.

Gereç ve Yöntemler: IDA ve BTM olarak değerlendirilen iki grup vardı. Beş kırmızı kan hücre parametresi [kırmızı kan hücresi (RBC), ortalama korpusküler hacim (MCV), hemoglobin (Hb), ortalama korpusküler hemoglobin (MCH), kırmızı kan hücresi dağılım genişliği (RDW)] çalışıldı ve on iki indeks [Menzier İndeksi (MI), Greenl ve King (GK), Kırmızı Kan Hücresi Dağılım Genişliği İndeksi (RDWI),Egland ve Fraser (EF), RBC, Sirdah İndeksi (SI) Ricarca İndeksi (R), Srivastava (S), Shine & Lal (SL), Ehsani (E), Kırmızı Hücre Dağılım Genişliği (RDW), 11T] hesaplandı.

Bulgular: On iki ayrıcı indeksden 11T indeksi, BTM'yı IDA'dan ayırmada en etkili olanıydı. 11T indeksi en yüksek Youden indeksine (% 69,8) ve doğruluğa (% 81,2) sahipti.

Sonuç: BTM'den IDA'yı ayırt etmede 11T indeksi en etkiliydi. RBC, GK, SI ve RDWI ise ayrım indeksleri yerine bir tarama aracı olarak kullanılabilir.

Anahtar kelimeler: Anemi; beta-talassemi; demir-eksikliği; eritrosit indeksleri

INTRODUCTION

Anemia is a condition in which the number of red blood cells or their oxygen-carrying capacity is insufficient to meet physiologic needs. It is a global public health problem affecting both developing and developed countries with major consequences for human health as well as social and economic development. Two most frequent types of microcytic anemia are iron deficiency anemia (IDA) and beta thalassemia minör (BTM). BTM is common in specific geographical areas, including the Mediterranean region (1,2). IDA is thought to be the most common cause of anemia globally (3). IDA occurs mainly as a result of inadequate intake, the menstrual cycle and gastrointestinal loss.

Microcytic anemia in case of thalassemia results from impaired globin chain synthesis and decreased hemoglobin (Hb) synthesis (4,5). Although distinct approaches are present in the treatment of both diseases, complete blood cell counts can not distinguish microcytic hypochromic anemia table. Diagnostic parameters for BTM and IDA are serum iron, ferritin and hemoglobin alpha 2 (HbA2) in electrophoresis (6). The measurement of HbA2 level is gold standard for the BTM diagnosis (7).

Although these tests are successful, they are time consuming, expensive, and not readily available to most people. Areas where thalassemia is endemic often have low health care resources and these assays may not be generally available.

Differentiating IDA from thalassemia carrier status is a frequent issue in medical practice, in particular in subjects with mild or moderate IDA and in regions where thalassemia is common. It is therefore clinically important to have a simple index that can effectively differentiate between IDA and thalassemia. It is not possible to distinguish both conditions using simple routine blood counts. Therefore, several simple screening indices have been developed for the differentiation of BTM and IDA (8-15).

Various red cell indices have been proposed in the past as easy and economical tools to discriminate BTM or IDA. However these indices have various accuracies reported in different studies (16-26).

In our study, we aimed to evaluate the discrimination power of twelve indices including; MI, GK, RDWI, EF, RBC, SI, R, S, SL, E, RDW, 11T.

MATERIALS AND METHODS

We retrospectively reviewed the laboratory data of patients with microcytic anemia (MCV<80 fl , MCH<27 pg and Hb <12 g/dL). The two group of patients were selected and included in the study; IDA and BTM. Patients with evidence of inflammatory disorders, malignancy, anemia consequent to chronic disease or a history of acute hemorrhage were excluded from this study.

C-reactive protein and liver function tests of all patients participating in the study were in the normal range.

The first group of 118 patients (98 females and 20 males) with a mean age of 35.6±14.9, had hemoglobin levels of less than 12 g/dl in women and 13 g/dl in men and serum ferritin levels in both genders were below 12 ng/ml. In addition, mean erythrocyte volume (MCV) and mean corpuscular hemoglobin (MCH) of patients were below 80 fl and 27 pg, respectively. This group was evaluated as the IDA.

The second group, which was evaluated as BTM, consisted of 91 patients (54 females and 37 males) with a mean age of 44.9±19.9. The criterion was that HbA2 levels should be over 3.5%. MCV and MCH values of this group were below 80 fl and 27 pg, respectively. However, serum ferritin levels were above 12 ng/ml. In this study group there was no patient having BTM and IDA together.

Serum ferritin levels of the patients were measured by the chemiluminescence method on the UniCel DXI 800 access (Beckman Coulter, USA) analyser. The complete blood counts were obtained in the autoanalyzer Cell-Dyn 3200 (Abbott, Germany). The HbA2 levels were determined using the high performance liquid chromatography (Trinity Biotech, Ireland).

In all groups, twelve indices (MI, GK, RDWI, EF, RBC, SI, R, S, SL, E, RDW, 11T) were calculated using the data obtained from whole blood counts such as Hb, RBC, MCV, MCH, RDW. The mathematical formulas of these indices are shown in Table 1.

The data were analyzed using SPSS software, version 21 (SPSS Inc., Chicago, IL, USA). The sensitivity and specificity of each index was calculated according to the cut-off values. The sensitivity, specificity, accuracy and Youden's index (YI) were calculated for each index as follows:

Sensitivity=[true positive /(true positive+ false negative)]×100,

Table 1. Mathematical formulas of the indices.

	INDEX	FORMULA	BTM	IDA
1	Mentzer Index(MI)	MCV/RBC	<13	>13
2	England and Fraser(EF)	MCV-RBC-(5*Hb)-5.19	<0	>0
3	Green and King İndeks(GK)	MCV2*RDW/100Hb	<72	>72
4	Red Blood Cell Distrubition Index (RDWI)	MCV*RDW/RBC	<220	>220
5	Red Blood Cell Count (RBC)	RBC	>5	<5
6	Sirdah Index (SI)	MCV-RBC-3*Hb	<27	>27
7	Ricarca Index (R)	RDW/RBC	<3,3	>3,3
8	Ehsani Index ((E)	MCV-10*RBC	<15	>15
9	Srivastava Index (S)	MCH/RBC	<3,8	>3,8
10	Shine and Lal Index (SL)	MCV2*MCH/100	<1530	>1530
11	Red Blood Cell Distrubition (RDW)	RDW	<14	>14
12	11 T*		>8	<8

The 11T index occurs when the results of the first 11 indices in the table are scored as " 0 " in favor of IDA and " 1 " in favor of BTM, and these scores are summarized.

Specificity=[true negative /(true negative+ false positive)]×100,

Youden's index=(sensitivity+specificity)−100,

Accuracy = (true negative + true positive)/(true negative + true positive + false negative + false positive)

RESULTS

Mean values, standard deviations (SD) of the hematological parameters in IDA and BTM groups are shown in Table 2. The twelve discrimination indices used in the evaluation are summarized in Table 3. 11T index was the most efficient in differentiating IDA from BTM and had the highest Youden's index (69.8%) and accuracy (81.2%). The other indices; RBC, GK, SI, RDWI, EF, R, MI, E were respectively following 11T index. But SL, S and RDW indices have lowerYouden's index and accuracy values.

Table 2. Hematological parameters in the BTM and IDA groups.

	IDA (mean±SD)	BTM (mean±SD)
RBC (1012/l)	4,58±0,5	5,59±0,6
Hb (g/dl)	10,01±1,5	11,34±1,2
MCV (fl)	69,46±7,1	63,31±4,1
MCH (pg)	21,81±3,6	20,37±1,5
RDW (%)	17,96±2,9	16,51±1,5
Ferritin (ng/ml)	3.98±2,2	96,4±166

Note: The results are shown as mean ± standard deviation (ranges).

Table 3. The 12 discrimination indices.

Index	Patient	Sensitivity (%)	Specificity (%)	Accuracy (%)	Youden's index
11T	IDA	75,8	94	81,2	69,8
	BTM	94	75,8		
RBC	IDA	86,8	82,2	80,9	69
	BTM	82,2	86,8		
GK	IDA	90,1	78,8	80,8	68,9
	BTM	78,8	90,1		
SI	IDA	75,8	92,4	80,5	68,2
	BTM	92,4	75,8		
RDWI	IDA	84,6	83	80,2	67,6
	BTM	83	84,6		
EF	IDA	74,7	92,4	80	67,1
	BTM	92,4	74,7		
R	IDA	81,3	81,4	77,8	62,7
	BTM	81,4	81,3		
MI	IDA	85,7	76,3	77,5	62
	BTM	76,3	85,7		
E	IDA	85,7	76,3	77,5	62
	BTM	76,3	85,7		
SL	IDA	97,8	10,1	51,6	7,9
	BTM	10,1	97,8		
S	IDA	21,3	84,7	50,7	6
	BTM	84,7	21,3		
RDW	IDA	1	94,9	45,9	-4,1
	BTM	94,9	1		

DISCUSSION

We tried to show the superiority of the separation powers of almost all indices suggested in the literature, which are used in IDA and BTM discrimination. Many studies have demonstrated high variation in the performance of discriminant indices. These indexes are 61-91% successful in properly classifying whether hypochromic and microcytic anaemia is due to BTM or IDA (6,8,16,17,18,20,22,24,25).

In our study; the 11T index showed good sensitivity, specificity, Youden's index and accuracy values of 94 %, 75.8%, 69.8% and 81.2%, respectively. Youden's index showed the following ranking with respect to the indices ability to distinguish between BTM and IDA: 11T > RBC> GK > SI> RDWI> EF > R> MI > E > SL > S> RDW.

We applied all eleven formulas and the index with the highest percentage for accurate diagnosis was 11T (81.2%) followed by RBC (80.9%), GK (80.8%), SI (80.5%), RDWI (80.2%), EF (80%), R (77.8%), MI (77.5 %), E (77.5%), SL (51.6%) S (50.7%), RDW (45.9%), respectively.

We also compared the results of other publications available in the literature with our work. In a study by Alexandre Janel et al. (19), 11 T indexes were proposed for the first time and demonstrated the efficiency of this new score with a high AUC (0.947), the highest Youden's index (83.2%), and accuracy (93%). However, in our work Youden's index and accuracy values were less than their values. Therefore, it is essential to determine cut-off of the indices for each laboratory for differentiation of IDA and BTM in a given population.

The RBC count is one of the most accurate indices available. In our study, the RBC count provided the best sensitivity (82.2%), specificity (86.8%), Youden's index (69%) and accuracy (80,9%). In the study by Jameel et al. (21), it was shown that RBC had a sensitivity of 88%, specificity of 90%, and Youden's index of 74%. Vehapoğlu et al. (18) demonstrated that RBC had an accuracy of 83.4%, while RDWI 's accuracy was 80%.

Okan et al. (20) showed RDWI sensitivity and specifcity were > 80% in the detection of BTM and IDA cases and Youden's index was 73,7%. Similarly, Beyan et al. (22), showed RDWI sensitivity of 87.9% and specifcity of 75.5% in the detection of BTM and IDA cases and Youden's index was 63,4%. Similarly, we found RDWI index sensivity as 83%, spesificity as 84.6%, Youden's index as 67.6% and accuracy as 80.2%. Similar observations showed percent accuracy of discriminant indices as: RDWI 92%, RBC 90%, GK 86%, EF 78%, MI 76%, S 67%, and SL 59% and RDW 59% (24).

In another study, Piplani et.al. (26) found that formula presented by Mentzer, Ehsani, Sirdah, RDWI, RBC and EF had good discriminative functions. Ntaios et al. (27) found that Youden's index and accuracy showed the following ranking with respect to the indices: GK, RBC, EF, RDWI, MI.

Various other red cell indices such as EF, and SL have been evaluated by the studies such as the one by Batebi et al. (28), showing a high sensitivity of 87.2% for the EF index in the diagnosis of BTM and thus, it was proposed as an acceptable discriminator between BTM and IDA. Plengsuree et al. (29) found that Youden's index and accuracy showed the following ranking: R, RDWI, GK, RBC, MI. In our study similarly we found efficiency of these indices as 11T, RBC, GK, SI, RDWI, EF, R, MI, E, SL, S, RDW respectively.

Obviously, a correct diagnosis in patients with microcytic anemia is important. Screening and accurate identification of hemoglobin (Hb) variants have become increasingly important in antenatal diagnosis and prevention of Hb disorders. It can provide an indication for avoiding unnecessary iron therapy in thalassemia carriers and for preventing severe and lethal forms of thalassemia syndromes.

Available laboratory hematologic analyses make it necessary to provide support and

interpretation for a correct clinical diagnosis. Therefore, several studies have derived discriminatory functions based on RBC indices that can be used to differentiate between patients with IDA and those with BTM.

Up to now, many investigators have used different mathematical indices to distinguish BTM from IDA using a complete blood count. This process helps to select appropriate individuals for a more detailed examination; however we think that differences in determining best equation and best cut-off value depends not only on the validity and reliability of laboratory tests that are performed in different situations and the accuracy of laboratory equipment, but also on genetic variations.

None of discriminant indices provides 100% sensitivity and 100% specificity for discrimination purposes. The numerous discriminant index studies reflects that researchers were continuously stimulated to devise new and supposedly better indices. In the last decade, multiple studies have been published and arrived in a conclusion that further confirmatory testing must be performed for a correct diagnosis. In some patients with hypochromic microcytic anemia it would be necessary to determine HbA2 and iron status for accurate diagnosis.

In conclusion, the proposed formulas provide an additional method to help distinguish BTM from IDA . Therefore, the proposed formulas need to be evaluated in future studies of larger population samples.

REFERENCES

1. 1. Olivieri NF. The beta-thalassemias.N. Engl. J. Med.1999;3 DOI: 10.1056/NEJM199907083410207.
2. Angastiniotis M, Modell B. Global epidemiology of hemoglobin disorders. Ann N Y Acad Sci. 1998;850:251–69.
3. http://www.who.int/vmnis/anaemia/prelence/en/
4. Warghade S, Britto J, Haryan R, Dalvi T, Bendre R, Chheda P et al. Prevalence of hemoglobin variants and hemoglobinopathies using cation-exchange high-performance liquid chromatography in central reference laboratory of India: A report of 65779 cases. J Lab Physicians. 2018;10(1):73-79.
5. Lukens JN. The thalassemias and related disorder: Anoverview. In: Lee GRet al.(eds). Wintrobe's Clinical Hematology 1999;405–33.
6. Thomas C, Thomas L. Biochemical markers and hematologic indices in the diagnosis of functional iron deficiency. Clin chem 2002;48(7):1066-76.
7. Mosca A, Paleari R, Ivaldi G, Galanello R, Giordano P. The role of haemoglobin A2 testing in the diagnosis of thalassaemias and related haemoglobinopathies. J Clin Pathol 2009;62:13-7.
8. Sirdah M, Tarazı I, Al Nassar E, Al Haddad R. Evaluation of the diagnostic reliability of different RBC indices and formulas in the differentiation of the beta-thalassaemia minor from iron deficiency in Palestinian population. Int J Lab Hematol 2008;30(4):324–330.
9. England JM, Fraser P. Discrimination between iron-deficiency and heterozygous thalassemia syndromes in differential diagnosis of microcytosis. Lancet 1979;20(1):145-8.
10. Mentzer Jr WC. Differentiation of iron deficiency from thalassaemia trait. Lancet 1973;1:882.
11. Srivastava PC. Differentiation of thalassaemia minor from iron deficiency. Lancet 1973;2:155–6.
12. Shine I, Lal S. A strategy to detect thalassaemia minor. Lancet 1977;1:692–4.
13. Bessman JD, Feinstein DI. Quantitative anisocytosis as a discriminant between iron deficiency and thalassemia minor. Blood 1979;53:288–93.
14. Ricerca BM, Storti S, d'Onofrio G, Mancini S, Vittori M, Campisi S,et al. Differentiation of iron deficiency from thalassaemia trait: a new approach. Haematologica 1987;72:409–13.
15. Green R, King R. A new red cell discriminant incorporating volum dispersion for differentiating iron deficiency anemia fromthalassemia minor. Blood Cells 1989;15:481–95.
16. Ullah Z, Khattak AA, Ali SA, Hussain J, Noor B, Bano R et al. Evaluation of five discriminating indexes to distinguish Beta-Thalassemia Trait from Iron Deficiency Anaemia. J Pak Med Assoc. 2016;66(12):1627-1631.
17. Huang TC, Wu YY, Chen YG, Lai SW, Wu SC, Ye RH et al. Discrimination index of microcytic anemia in young soldiers: a single institutional analysis.Plos One 2015;13:1-10.
18. Vehapoglu A, Ozgurhan G, Demir AD, Uzuner S, Nursoy MA, Turkmen S et al. Hematological indices for differential diagnosis of Beta thalassemia trait and iron deficiency anemia. Anemia 2014;2014:1-7.
19. Janel A, Roszyk L, Rapatel C, Mareynat G, Berger MG, Serre-Sapin AF. Proposal of a score combining

red blood cell indices for early differentiation of beta-thalassemia minor from iron deficiency anemia. Hematology 2011;16(2):123-7.

20. Okan V, Cigiloglu A, Cifci S, Yilmaz M, Pehlivan M. Red cell indices and functions differentiating patients with the beta-thalassaemia trait from those with iron deficiency anaemia. J Int Med Res 2009;37(1):25-30.

21. Jameel T, Baig M, Ahmed I, Hussain MB, Alkhamaly MBD. Differentiation of beta thalassemia trait from iron deficiency anemia by hematological indices. Pak J Med Sci 2017;33(3):665-669.

22. 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(6):524-6.

23. Hafeez Kandhro A, Shoombuatong W, Prachayasittikul V, Nuchnoi P. New Bioinformatics-Based Discrimination Formulas for Differentiation of Thalassemia Traits From Iron Deficiency Anemia. Lab Med 2017;48(3):230-237.

24. Demir A, Yarali N,Fisgin T, Duru F,Kara AR. Most reliable indices in differentiation between thalassemia trait and iron deficiency anemia. Pedia Int 2002;44(6):612-6.

25. Kumar A,Saha D,Kini J, Murali N, Chakraborti S, Adiga D. The role of discriminant functionsin screening beta thalassemia traitand iron deficiency anemia among laboratory samples. Journal of Laboratory Physicians 2017;9:195-201.

26. PiplaniS, Madan M, Mannan R, Manjari M, Singh T, Lalit M. Evaluation of Various Discrimination Indices in Differentiating Iron Deficiency Anemia and Beta Thalassemia Trait: A Practical Low Cost Solution. Annals of Pathology and Laboratory Medicine 2016;03(06):552-59.

27. Ntaios G, Chatzinikolaou A, Saouli Z, Girtovitis F, Tsapanidou M, Kaiafa G et al. Discrimination indices as screening testsfor ®-thalassemic trait. Ann Hematol 2007;86:487–491.

28. Batebi A, Pourreza A, Esmailian R. Discrimination of ®-thalassaemia minor and iron deficiency anemia by screening test for red blood cell indices. Turk J Med Sci 2012;42:275-80.

29. Plengsuree S, Punyamung M, Yanola J, Nanta S, Jaiping K, Maneewong K et al. Red Cell Indices and Formulas Used in Differentiation of b-ThalassemiaTrait from Iron Deficiency in Thai Adults. Hemoglobin 2015;39(4):235-9.