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Cut off Determination of Discrimination Indices in Differential Diagnosis between Iron Deficiency Anemia and β- Thalassemia Minor

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ABSTRACT

Objective: The two most frequent hypochromic microcytic anemias are β- thalassemia minor (BTM) and iron deficiency anemia (IDA). Several discrimination indices have been proposed to quickly discriminate these similar entities via parameters obtained from automated blood count analyzers. The aim of this study to evaluate the diagnostic reliability of ten discrimination indices in the differentiation of Iron deficiency anemia (IDA) from β Thalassemia Minor (BTM).

Methods: This study was conducted on 100 BTM and 70 cases with IDA in southern Iran. This evaluation was conducted through calculation sensitivity, specificity, positive and negative predictive value, Likelihood ratio positive, likelihood ratio negative and also we recalculated cut-off values for every formulas in our population and determination of Area Under Curve related to receiver operative characteristic (ROC) curves.

Results: ROC for each discrimination indices show that, the highest diagnostic value based on the area under the ROC curve are related to the Green & King, England & Frazer and then Sirdah formulas (0.909, 0.907, 0.904, respectively) in South-east of Iran, and relatively different cut-off values for every formula.

Conclusion: The spectrum β thalassemia mutations in each population can affect on various RBC indices, therefore, it is suggested to determine cut-off value for every formula in different populations.

KEY WORDS: Anemia; Discrimination formulas; β-thalassemic trait. Iron deficiency; Microcytosis; Cutoff

INTRODUCTION

β Thalassemia Minor (BTM) and Iron deficiency anemia (IDA) are the most commonly encountered with mild hypochoromic microcytic anemia. The differential diagnosis between IDA and BTM is an important concern for every physician; to avoid unnecessary iron therapy and false diagnosis of BTM, especially in pre-marriage counseling toward prevention of β thalassemia (β thal) major baby birth and minimization of expense. Prevention of β thalassemia (thal) major is one of the most important programs of the health system especially, in countries with high β thal gene prevalence. Screening of carriers and counseling at risk couples are the most successfully approaches in reduction of new cases β thal major in the world.[1]

BTM and IDA present similar pattern as hypochromic microcytic anemia and it's not possible to differentiation them only by red blood cell parameters. Definitive methods for differential diagnosis between BTM and IDA include quantitative detection of HbA2 and DNA mutations

analysis. While being accurate, these tests are too expensive and time-consuming for initial mass screening.

Various formulas have been proposed according to the index of red blood cells.[2-12] All these formulas have been tested with different cut-off values but none has been found to be sufficiently specific or sensitive, which could be associated with mutations in each region. Despite multiple mutations in the β globin gene, usually a fair number of different mutations usually account for the most of the thal patients in each area. On the other hand, each mutation affects a certain amount of globin chain synthesis, and makes changes accordingly red cell indices.

About 25,000 transfusion-dependent thal patients who have been registered by Iranian Blood Transfusion Organization.[13] The gene frequency of β-thal is varying considerably in different area of Iran, the highest rate with more than 10% is reported around the Caspian Sea and the Persian Gulf.[14] Sistan and Balouchistan, the largest province (about 181,785 km2), is located in southeastern Iran in a subtropical and malaria-endemic area. So far, 2500 β thal major patients hold the highest rate of β thal major cases in the country[15] and beta gene frequency is vary 6.2 to 12.9%.[16] Given the high prevalence of IDA and BTT, geographical distribution of towns and villages in Sistan and Balouchistan, lack of access to specialized laboratory facilities in some cities and costly of tests led to we looking for a simple and additional reliable approach. Therefore, reliable differentiation formulas, which are calculated on RBC indices that easily obtain by cell counting devices, facilitate the diagnosis process. At present, cell counters are widely used in routine practice, so screening can be done without additional costs to medical systems. (Successful prevention programs for BTT in Greece and Italy have relied on screening by RBC indices and HbA2 concentration.[17]

The aim of this study was to compare the diagnostic value of the formula that computed on red blood cells indices and assessment cut-off point of them, and determine the most reliable formula in the Sistan and Balouchistan population.

MATERIALS AND METHODS

In this cross-sectional study, we selected 77 IDA and 100 BTM among subjects of the obligatory premarital test screening program for BTM who referred to the reference laboratory of Zhedan University of Medical Sciences in the southern Iran. IDA was defined as a microcytic anemia (mean corpuscular volume [MCV], <80 fL; with a con-current serum ferritin levels < 12 μg/dl. BTM was defined as microcytosis, hemoglobin A2 ≥ 3.5% and having identified a mutation.

The complete blood count (CBC) was measured by an automated hematology analyzer (Sysmex K1000; Sysmex, Tokyo, Japan).

In this study, we compared ten different discrimination formulas with respect to seven parameters include sensitivity, specificity, positive predictive value, negative predictive value, Likelihood ratio positive , likelihood ratio negative and also evaluation of receiver operative characteristic (ROC) curves. In addition, for each formula we calculated the new cutoff value with the highest accuracy (the point that sum of sensitivity and specificity is the most) in our population.

The diagnostic value of each formulas was performed by calculating the following indices; Specificity: True negative / (true negative + false positive), Sensitivity: True positive /(true positive + false negative), Positive predictive value: True positive / (true positive + false positive), Negative predictive value: True negative/ (true negative + false negative), Likelihood Ratio positive: sensitivity / (1- Specificity), Likelihood Ratio Negative: (1-Sensitivity)/specificity).

RESULTS

This study was conducted on 100 patients with BTM and 77 subjects with IDA. The mean and standard deviation of the various hematological parameters of IDA and BTM groups are shown in Table 1.

The sensitivity, specificity, positive and negative predictive value, Likelihood ratio positive, likelihood ratio negative and the characteristic (ROC) curves receiver operative in connection with each formula and Published and proposed cut off for differential diagnosis of IDA and BMT are shown in Table2.

PPV, Positive predictive value; NNP, Negative predictive value; LR+, Likelihood Ratio positive; LR-, Likelihood Ratio Negative; AUC, Area Under the Curve; CI, confidence interval.

Receiver operative characteristic curve (ROC) for each of the calculations is shown in Figure 1. As in Table 2 and Figure 1 are shown, the highest diagnostic value based on the area under the ROC curve are related to the Green & King, England & Frazer and then Sirdah formulas.

Table 1: Mean various hematological parameters in β Thalassemia Minor (BTM) and Iron Deficiency Anemia (IDA)

Parameters	BTM	IDA
	Mean ± SD	Mean ± SD
RBC (10^{12}/L)	7.1±0.5 *	4.8±0.7
Hb (g/dl)	12.8±1.2*	10.8±1.3
Hct (%)	43±4*	34.7±3.5
MCV (fl)	61±3.1*	72.3±5.8
MCH (pg)	18.1±1*	22.5±3
MCHC(g/l)	29.7±1.4	31±2.2
RDW	16.2±1.9	16.2±2.7

Significant (P<0.05)

Table2: Evaluation of different red blood cell mathematical formulas in differentiation of β Thalassemia Minor (BTM) from Iron Deficiency Anemia (IDA)

Index	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	LR+	LR-	Published Cut off for BTT	Proposed Cut off for BTT (our population)	AUC 95% CI
Green & King MCV^2*RDW/100Hb	84	84	84	84	5.4	0.18	<72	<66.5	0.909 0.862-0.956
England & frazer MCV-RBC-(5HB)-3.4	67	90	94	53	6.9	0.36	<0	<6.5	0.907 0.859-0.954
Sirdah MCV-RBC- (3Hb)	73	85	88	68	4.8	0.31	<27	<29	0.904 0.855-0.952
Kerman2 KERMAN 1*10/MCHC	79	68.2	60.8	84	2.5	0.30	<85	<90	0.823 0.754-0.893
RDW idex MCV*RDW/RBC	72	79	82	68	3.4	350.	<220	<212	0.819 0.747-0.891
Mentzer MCV*MCH/RBC	72	82	68	67	4.1	0.33	<13	<13	0.819 0.747-0.891
Ehsani MCV-(10 RBC)	71	82	58	66	4	0.34	<15	<16	0.817 0.746-0.889
Kerman 1 MCV*MCH/RBC	69	64	58	74	1.9	0.47	<300	<280	0.738 0.714-0.823
Shin & Lal MCV^2*MCH/100	64	57	44	75	1.5	620.	<1530	<1004	0.709 0.623-0.796
Srivastava MCH/RBC	64	57	44	74	1.5	0.62	<3.8	<4.1	0.696 0.606-0.786

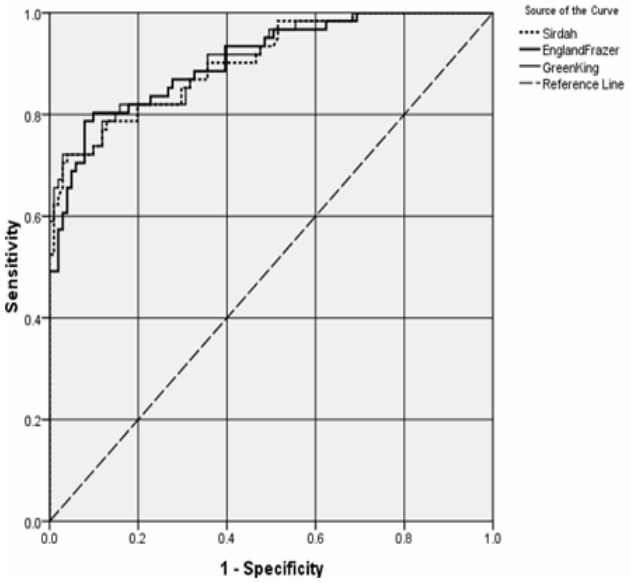

Figure 1: Receiver operative characteristic curves (ROC) of Green–King, England & Frazer, and the Sirdah formulas (P value for each formula < 0.001)

DISCUSSION

IDA and BTM are the most common cause of microcytic anemia. In order to reduce the cost, time and complicated procedures for their discrimination, various RBC indices and formulas have been used. It is important to select which formula is more accurate in the differentiation of BTM from IDA cases. The most of BTM cases are asymptomatic and without specialized tests may be missed or sometimes misdiagnosed as IDA. In our previous study we find un-warming of the carrier state in premarital screening tests were the most important cause of new beta thal major case birth in Sistan and Balouchistan province.[18] Hematologic parameters will not normalize in BTM after iron therapy. This study shows that the hematological parameters include; Hb, HCT, MCV and MCH in BTM significantly are lower than IDA, and RBC count had significantly higher compare to IDA. In BTM cases reduction in MCV and MCH value did not correlate with the degree of anemia, similar trend of findings also reported.[5, 19] In BTM population, there is a relationship between the hematologic phenotype and the type of β globin gene mutation[20] and, also between MCV and type of the thal mutation was reported a significantly correlation.[21] These data could explain the observed differences in many studies and the need to define new cut-off values for each population. Sistan and Balouchistan population is a homogenous population (about 70% of couples had consanguineous marriages) had and more than 75% of BTM have IVS 1-5 mutation.[15, 22]

Sensitivity and specificity of these formulas have been evaluated in several studies, but none have had 100% sensitivity and specificity. However, Last studies have shown that these formulas properly identify only 61–91% of the patients with microcytic anemia due to BMT or IDA. As each formula has showed overlapping values in these patients , none of them was completely satisfactory in differentiating between BMT and IDA.[10, 23-32]

Evaluation of different formulas in present study was performed, and compared according to the ROC curve.

The results show that respectively Green & King, England & Frazer and then Sirdah formula have the largest AUC (0.909).(Table 2).

Natio et al., evaluated seven differentiation indices and find that Green & King had the highest sensitivity with 75% and then the England & Fraser, RBC, RDW, Mentzer indices and none of them cannot be relied on for safe differential diagnosis.[33] Sirdah et al., conducted a study in 2007 to evaluate the reliability of different RBC indices and their formulas (MCV-RBC-3.Hb) in Palestinian population and concluded that their formulas (Sirdah), Green & King and index RDW have the highest accuracy in differentiating between the BTM and IDA.[9] Demir et al., evaluated eight discrimination indices and concluded that none of the discrimination indices showed 100% sensitivity and specificity. RBCs and RDWI could differentiate BTM from IDA in 90% of the patients with RBC and 92% with RDWI.[11]

Matos et al., evaluated discrimination power of seven indices to differentiate between IDA and BTM in Brizilian population and reported Green & King and RDWI showed the highest reliability, as they had the largest AUCs (0.919, 0.912, respectively).[34] Shen et al., evaluated the validity of 12 indices in differentiation between IDA and BTM for Chinese children. Green and King Index showed the highest reliability (AUC=0.947) and Srivastava and Shine & Lal Index the lowest reliability (AUC=0.679 and 0.532, respectively).[35]

It can conclude that Green & King, England & Frazer and then Sirdah formulas, appear to be reliable and can be used for differentiating of BMT from IDA in South-east of Iran. So microcytic hypochromic anemia cases could be easily screened out in mass screening in the absent of other improve procedures. Should be considered, none of these indices shows the sensitivity and specificity of 100%.

The spectrum thalassemia mutations in each population can affect on of various RBC indices, as a result, the cutoff point of discrimination formulas varies. Therefore, it is suggested to determine the cut-off point for every formula in different populations.

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