



The Evaluation of Results of Twenty Common Equations for Differentiation of Beta Thalassemia Trait from Iron Deficiency Anemia: A Cross-Sectional Study

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Abstract

Background: Beta Thalassemia Trait (BTT) and Iron Deficiency Anemia (IDA) were two common clinical problems with clinical hypochromic and microcytic manifestations, and their differentiation from each other was very important and needs innovative formulas and laboratory tests. Since the consideration of anemia as a pair with BTT leads to beta-thalassemia major birth in 25% of cases, offering prospective parents detailed information about the likelihood of their offspring developing BTT is essential. The present study aimed to investigate the performance of common equations in differentiation of BTT from IDA.

Methods: In the present cross-sectional study, twenty common equations were selected in the differentiation of BTT from IDA. To evaluate the equations, the tests of 292 individuals (73 individuals with BTT and 219 individuals with IDA) were compared with the initial diagnosis of hypochromic and microcytic anemia using the formulas. Descriptive and value indices and Roc curve were utilized for all equations to analyze the results.

Results: Among twenty differential equations, Bordbar, Kerman I, II and Srivastava equations had the highest area under Roc curve (AUC) of 0.841, 0.838, 0.836, and 0.830 respectively, but Kandhro I. equation had the lowest AUC (0.378).

Conclusion: Given the importance of AUC and value indices of differential equations in clinical decision making, and results of evaluating common equations in differentiation of BTT from IDA. It is essential to improve the values of the equations by re-examining the parameters involved in them.

Keywords: Red blood cell; Thalassemia; Anemia; Mentzer index; Differentiation equation

Introduction

Thalassemia is an inherited blood disorder in which the survival of red blood cells decreases

due to the imbalance in the production of beta globulin chain. This disorder has two forms, mi-



nor (Beta Thalassemia Trait (BT^T) and major, among which the latter needs urgent treatment (1). Its prevalence in Iran is more than 10% for cities around the Persian Gulf and the Caspian Sea, and 4%-8% in other cities of Iran and 2% in West Azerbaijan Province (2,3). Iron deficiency anemia (IDA) is a common clinical problem defined by WHO as hemoglobin of less than 12 and 13 gr per deciliter for adult men and women respectively (4). The prevalence of iron deficiency anemia varies in different cities of Iran and has been reported from 21.5% to 42.7% (5).

Beta thalassemia is the most common type of hereditary and monogenic hemoglobinopathy. Methods of diagnosis of hemoglobinopathies include High-Performance Liquid Chromatography, Hemoglobin Electrophoresis, Screening of PCR Mutations, and DNA tests which are all costly, require sophisticated equipment and skilled workforce. Therefore, it is not possible to access them in any city (6). Since 25% of infants of male and female individuals with BT^T are born with beta thalassemia major, the lack of diagnosis and screening increases the number of infants with beta thalassemia major; and these infants will have severe anemia and Hepatosplenomegaly and will need serious medical interventions such as Regular Transfusion Program and Chelation Therapy in at least the first two years of life (7). Therefore, offering prospective parents detailed information about the likelihood of their offspring developing BT^T is essential.

A beta thalassemia major prevention plan has been implemented throughout Iran since 1998. According to the plan, the couple specifies their health in terms of having the BT^T by testing their blood before marriage. If the test of both individuals is less than 80 fl in terms of the Mean Corpuscular Volume (MCV) and less than 27 Pg in terms of the Corpuscular Mean of Hemoglobin (MCH) (both of them with microcytic hypochromic anemia), they are referred to the Beta Thalassemia counseling center of the health center; and the complementary and differentiating tests indicate that their anemia is due to the BT^T or IDA (8,9). BT^T and IDA are two most common microcytic hypochromic anemia. These two

types of anemia have similar manifestations and can be differentiated from each other by laboratory tests and though blood indices. Diagnosis of the IDA is performed by testing iron metabolism such as serum iron, Total Serum Iron-Binding Capacity, and measuring the amount of serum ferritin. However, the diagnosis of BT^T is performed based on the HPLC (High-Performance Liquid Chromatography) and Hemoglobin A2 (HbA₂) level (over 3.5%). However, the laboratory diagnosis and differentiation of the BT^T from IDA requires advanced equipment and high cost (10).

There are different equations for differentiation of the BT^T from IDA, including equations by Mentzer (Men), Ehsani (Eh), England & Fraser (EF), Green & King (GK), RBC counts, RDW, RDWI, Ricerca (Ric), Shine & Lal (Sh -L), Sirdah (Sir), Sirvastava (Sriv), and M/H Ratio (10,11). Each of these differentiators have different sensitivity (SEN), specificity (Spe), and value indices; and different papers have reported different results (12-15).

The innovation of the present study is the simultaneous comparison of 20 differential equations. Many related research studies have used a few numbers equations than our study to diagnose beta-thalassemia from iron deficiency anemia. Moreover, the present study was the first study in the region.

The present study aimed to evaluate the ability of 20 differential equations consisting of simple blood parameters in the separation of individuals with the BT^T from those with the IDA by calculating the sensitivity, specificity, Youden's index, and other value indices and according to the Roc Curve.

Materials and Methods

Source of Equations

The present study was cross-sectional research that investigated articles published in databases, namely PubMed, Scopus, ProQuest, Web of Science and Google Scholar and selected 20 equations for the differentiation of BT^T from IDA by

considering the possibility of acquiring blood indexes of differential equation in terms of laboratory and its breadth.

Data Collection

The data of study was obtained from cases of couples who participated in a project for the prevention of beta-thalassemia major and underwent blood sampling for BTT screening before marriage since 2012-2019 (700 couples). This is the process of identifying couples. First, the man's blood counts are tested. If Mean Corpuscular Volume (MCV) and Corpuscular Mean of Hemoglobin (MCH) of male were more than 80fl and 27pg, the couple was excluded from the beta-thalassemia diagnosis process (408 samples).

Otherwise, the woman's blood counts will also be tested. If the result of testing both individuals is as the MCV of less than 80fl and MCH of less than 27Pg (both with microcytic hypochromic anemia) (292 samples) (7), the couple is referred to the Beta Thalassemia counseling center of health center in Khoy city (West Azerbaijan, Iran) (Fig. 1). A survey of the archive of the center revealed that 292 couples were both hypochromic and microcytic anemia. Exactly 73 individuals had BTT and 219 had IDA according to complementary and genetic testing (amplification refractory mutation system (ARMS)-PCR). For original data, please contact corresponding author.

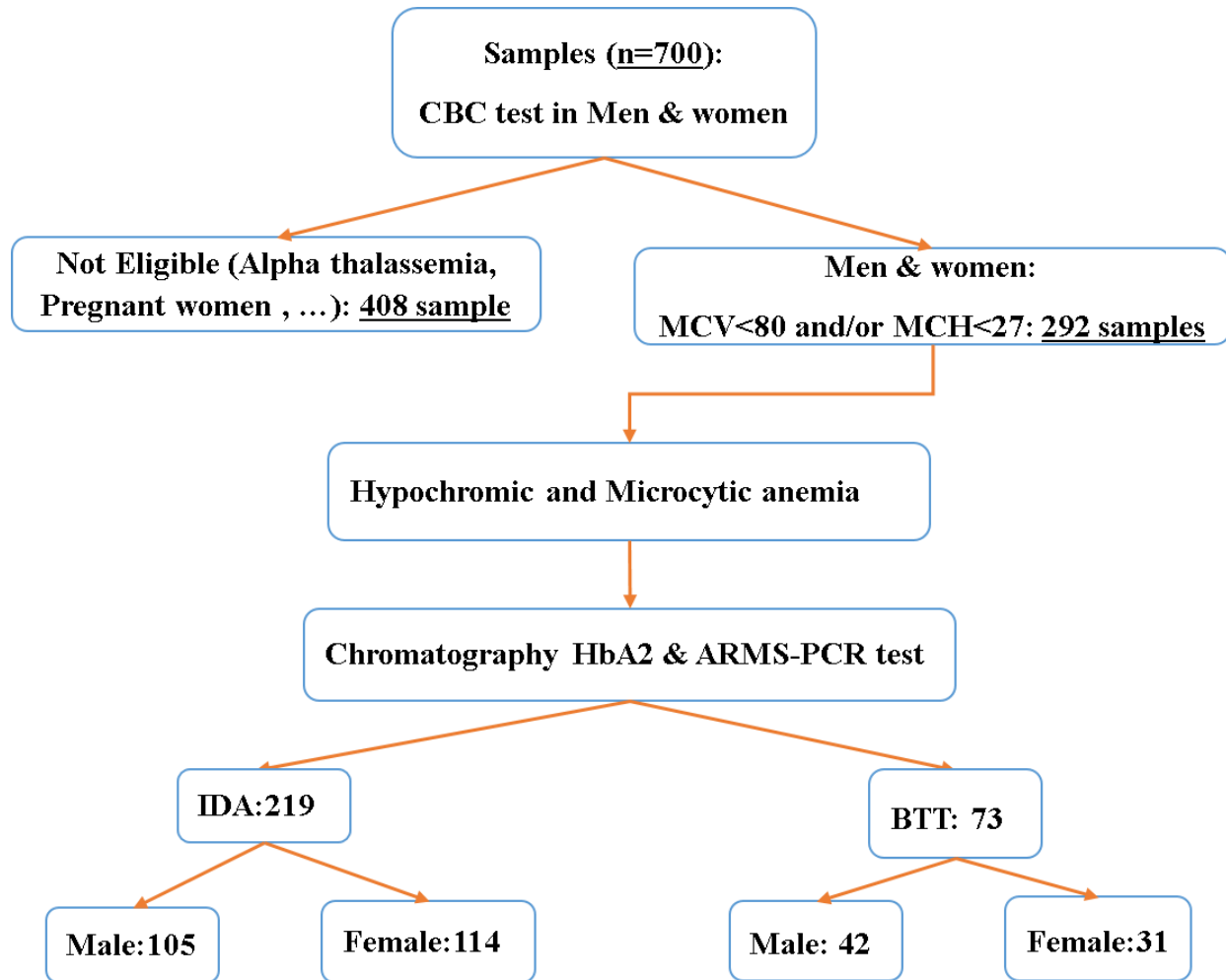


Fig. 1: The study selection flow diagram

Inclusion and Exclusion criteria

The individuals with alpha thalassemia during the genetic testing were excluded from the study since the study was on differential indices of the BTT. There were also no pregnant women among the couples.

Ethical Approval

The protocol of study was also reviewed and approved by the Ethical Committee of the Research Deputy of Khoy University of Medical Sciences, Khoy, Iran. Informed consent had been taken from all patients.

Statistical Analysis

To evaluate and compare each of the equations, the method of calculation and their numerical values were extracted for assigning to the BTT. Every equation is bivariate (BTT or IDA). Table 1 mentions cutoff points for BTT. The values of sensitivity, specificity, and Youden's index (Y.I) and other value indices were also calculated as Table 2.

Table 1: Equation of differentiation of BTT from IDA along with reference and cutoff point of the BTT

ID	Discriminant-Formula	Formula(Cut off)	ID	Discriminant Formula	Formula(Cut off)
1	Matos-Carvalho (M&K) (13)	$(1.91 \times RBC) + (0.44 \times MCHC) (>23.85)$	11	Green&King (G&K) (24)	$(MCV^2 \times RDW) / (Hb \times 100) (<72)$
2	Srivastava(Sriv) (16)	$MCH / RBC (<3.8)$	12	Kandhro I (Kan.I)(25)	$(RBC / HCT + 0.5 \times RDW)$ (cutoff<8.2) (17)
3	Shine&Lal (SH&L) (17)	$MCV^2 \times MCH^3 / 01 (<1530)$	13	Kandhro II(Kan.II) (25)	$RDW \times 5 / RBC (<16.8)$
4	KermanI (Ker.I) (18)	$MCV \times MCH / RBC (<300)$	14	Sirdah (26)	$MCV - RBC - (3 \times Hb) (<27)$
5	KermanII(Ker.II) (18)	$Ker.I \times 10 / MCHC (<85)$	15	Sirachainan (Sir) (27)	$1.5 \times Hb - 0.05 \times MCV$ (cutoff>14)
6	RDW (19)	$MCV \times RDW / RBC (<220)$	16	Bordbar (28)	$(80 - MCV \times 27 - MCH) (>44.76)$ $(Hb \times RDW \times 100) / (RBC^2 \times MCHC)$ (<21)
7	Mentzer (Men) (20)	$MCV / RBC (<13)$	17	Keikhaei(Kei) (29)	
8	Ricerca(Ric) (21)	$(RDW / RBC) (<3.3)$	18	Huber-Herklotz (H&H)(30)	$(MCH \times RDW \times 0.1 / RBC) + RDW$ (cutoff<22) (23)
9	Ehsani (Eh) (22)	$MCV - (10 \times RBC) (<15)$	19	Zaghloul(Z.I) (31)	$Hb + Hct + RBC$ (Males>55.7, Females > 51.6)
10	England&Fraser(E&F) (23)	$MCV - RBC - (5 \times Hb) - 3.4 (<0)$	20	Zaghloul(Z.II) (31)	$Hb + Hct + RBC - RDW$ (Males>40.4, Females> 35.6)

Table 2: Definition of basic Indices

ID	Index (abbreviation)	Formula
1	Sensitivity (SEN)	$TP / (TP + FN) \times 100$
2	Specificity (Spe)	$TN / (TN + FP) \times 100$
3	Positive Likelihood Ratio (PLR)	$SEN / (1 - SPE)$
4	Negative Likelihood Ratio (NLR)	$(1 - SEN) / SPE$
5	Youden Index (Y.I)	$(SEN + SPE) - 1$
6	Accuracy (Acc)	$(TP + TN) / (TP + TN + FP + FN)$
7	Positive Predictive Value (PPV)	$TP / (TP + FP) \times 100$
8	Negative Predictive Value (NPV)	$TN / (TN + FN) \times 100$

The normality of the variables was evaluated by Kolmogorov-Smirnov test. Accordingly, Mann-Whitney (Non-parametric) test (*P*-value) for MCV(<0.001), MCH(<0.001), HbA2(<0.001) and RDW (<0.001) and t-test for RBC(0.063), HGB(0.199), HCT(0.287) and MCHC (0.217) was performed (significance level=0.05). SPSS v.20 (IBM Corp., Armonk, NY, USA) was used for statistical analysis of data and drawing the Roc curve and reporting the area under the curve (AUC).

Results

Descriptive analysis

Data of 292 individuals, including 147 men and 145 women (73 individuals with the BTT including 42 men and 31 women) were analyzed; and the individuals' mean (sd) age was 24.56 (6.02) years. Table 3 reported the demographic characteristics of patients. Table 4 indicated description of blood characteristics for overall, BTT and IDA as well as sex groups along with the significant difference between descriptive indices of two groups using the t-test or Mann-Whitney (Non-parametric) test.

Table 3: Demographic characteristics of study samples

Variable		Frequency (%)		Total (%)
		BTT: 73(25)	IDA:219(75)	
Sex	Male	42(28.58)	105(71.42)	147(50.34)
	Female	31(21.37)	114(78.63)	145(49.66)
Education	Primary	35(26.92)	95(73.08)	130(44.52)
	Secondary	25(25.00)	75(75.00)	100(34.25)
	University	13(20.97)	49(79.03)	62(21.25)
Family marriage	Yes	13(32.50)	27(67.50)	40(13.70)
	No	60(23.81)	192(76.19)	252(86.30)
Residents	City	20(13.33)	130(86.67)	150(51.37)
	Village	53(37.32)	89(62.68)	142(48.63)

Table 4: Mean ± standard deviation of blood-related indices of the overall, BTT & IDA as well as sex groups.

Index	Overall	BTT				IDA				P
		Males	Female	P	Overall	Males	Females	P	Overall	
RBC	5.63±0.71	6.44±.83	5.71±.43	<0.001	6.13±.77	5.91±.46	5.09±.49	<0.001	5.48±.63	<0.001
HGB	13.4±1.91	13.25±1.11	11.51±1.02	<0.001	12.51±1.37	15.07±1.52	12.52±1.48	<0.001	13.74±1.97	<0.001
HCT	42.23±4.58	43.89±3.34	38.20±2.81	<0.001	41.46±4.20	45.60±3.42	39.63±3.75	<0.001	42.48±4.67	0.112
MCV*	75.11±7.10	68.28±6.67	67.25±4.83	0.772	67.83±5.93	77.45±4.91	77.59±6.33	0.453	77.52±5.69	<0.001
MCH*	23.99±3.19	20.69±2.54	20.41±2.15	0.877	20.57±2.37	25.62±2.62	24.68±2.45	0.001	25.13±2.57	<0.001
MCHC	31.76±2.12	30.20±1.27	30.15±1.67	0.864	30.18±1.44	33.01±2.08	31.59±1.79	<0.001	32.26±2.06	<0.001
RDW*	14.70±2.46	15.65±1.73	15.88±1.50	0.487	15.75±1.63	14.15±3.00	14.58±2.14	0.003	14.37±2.59	<0.001
HbA2*	3.17±1.39	5.16±.90	4.93±1.28	0.340	5.06±1.07	2.68±.88	2.42±.66	0.004	2.53±.78	<0.001

P: *P*-value *: Mann-Whitney test of Means and others t-test

Analytical analysis

Furthermore, values of SEN, SPE, PPV, NPV, LR+, LR-, AUC and YI were reported for 20 dif-

ferential equations (Table 5). The final criterion for selecting the best differential index was the size of the area under the ROC Curve (AUC).

Table 5: Diagnostic values twenty differential indices.

<i>ID</i>	<i>Index</i>	<i>AUC(95% CI)</i>	<i>Sen</i>	<i>Spe</i>	<i>PPV</i>	<i>NPV</i>	<i>PLR</i>	<i>NLR(%)</i>	<i>Acc</i>	<i>YI</i>
1	Bor	0.841(0.780-0.902)	0.791	0.885	0.695	0.927	6.903	0.235	0.862	0.676
2	Ker.I	0.838(0.780-0.896)	0.843	0.834	0.621	0.943	5.103	0.188	0.836	0.677
3	Ker.II	0.836(0.772-0.899)	0.765	0.907	0.722	0.924	8.258	0.259	0.873	0.672
4	Sriv	0.830(.769-.894)	0.786	0.885	0.687	0.927	6.851	0.242	0.861	0.671
5	Eh	0.821(0.759-0.882)	0.814	0.834	0.612	0.933	4.930	0.222	0.829	0.649
6	Men	0.788(0.725-0.851)	0.814	0.770	0.532	0.928	3.550	0.240	0.781	0.584
7	Sh-L	0.780 (0.725-.842)	0.888	0.669	0.470	0.948	2.691	0.165	0.724	0.558
8	Sirdah	0.683(0.610-0.756)	0.691	0.674	0.398	0.875	2.122	0.457	0.678	0.365
9	Kei	0.630(0.560-0.700)	0.897	0.362	0.308	0.917	1.407	0.283	0.491	0.259
10	RDW	0.586(0.513-0.659)	0.911	0.260	0.280	0.903	1.232	0.338	0.416	0.172
11	G-K	0.577(0.504-0.651)	0.926	0.227	0.275	0.907	1.199	0.322	0.395	0.154
12	E-F	0.570(0.493-0.647)	0.647	0.495	0.285	0.818	1.282	0.712	0.531	0.142
13	Kan.II	0.556(0.482-0.630)	1	0.111	0.262	1	1.125	0	0.325	0.111
14	Ric	0.556(0.482-0.630)	1	0.111	0.263	1	1.125	0	0.325	0.111
15	Matos	0.544(0.467-0.621)	0.808	0.282	0.262	0.824	1.127	0.676	0.408	0.091
16	Huber	0.520(0.442-0.598)	0.794	0.246	0.250	0.791	1.053	0.835	0.378	0.040
17	Z.II	0.512(0.434-0.590)	0.926	0.097	0.245	0.807	1.026	0.752	0.296	0.024
18	Z.I	0.510(0.432-0.588)	0.941	0.082	0.242	0.818	1.025	0.712	0.286	0.023
19	Sir	0.420(0.339-0.501)	0.691	0.151	0.203	0.611	0.814	2.040	0.279	- 0.157
20	Kan.I	0.378(0.297-0.459)	0.602	0.153	0.183	0.550	0.712	2.586	0.261	- 0.243

M:Males; F:Females

Based on findings and the AUC (Fig. 2), Bor, Ker.I, Ker.II, Sriv and Eh differential equations had the highest precision and performance (high-

er value indices); and the RDW, GK, Kan.II, Ric, Matos, Huber, Z.I, Z.II, and Kei equations had high sensitivity, but low specificity.

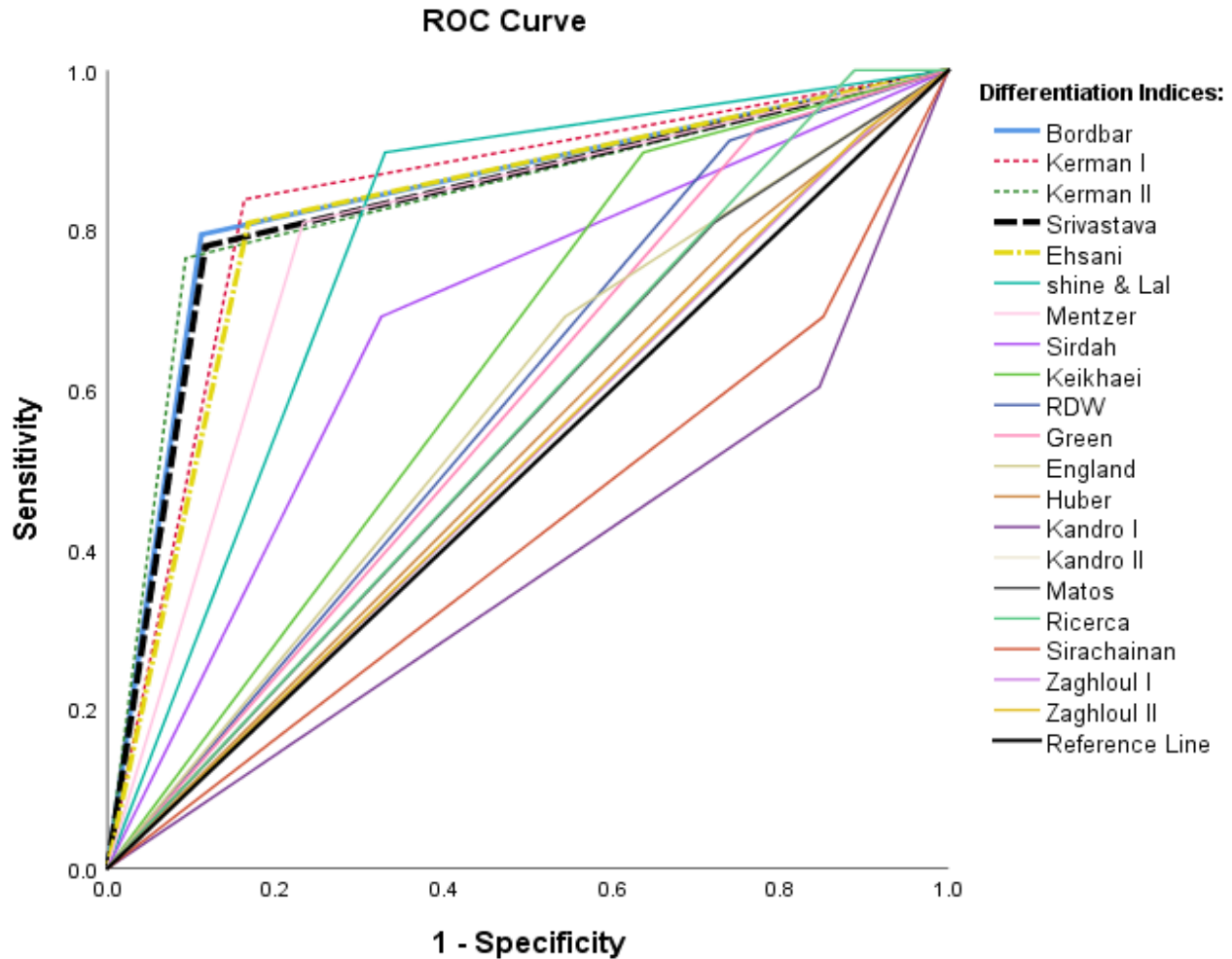


Fig. 2: Roc Curves of blood indices

Discussion

The present study aimed to evaluate the ability of 20 equations of differentiating BTT from IDA including simple blood indices. Sensitivity, specificity, Youden's index, and other value indices, and Roc curve drawing were used for evaluation. Findings of the present study indicated that blood indices, namely RBC, RDW and HbA2 in individuals with BTT, and indices, namely HGB, HCT, MCV, MCH and MCHC in individuals

with IDA were higher than the comparison group, but the differences were statistically significant only for HbA2, MCHC, HGB and RBC. Examination of the research findings based on gender also indicated that blood indices of men and women with IDA were similar, but among individuals with the BTT, men had higher RBC and HbA2 than women and the difference was significant (Table 4).

In a similar study (12), only the MCHC index was higher in individuals with BTT; and the differ-

ence of the HGB index was not significant between two groups. In another study (15), RBC, HGB, HbA2 and MCV indices were greater in individuals with Beta Thalassemia Trait; and the MCH and RDW were greater in those with IDA, but the study did not report any significant difference. Matos et al. (13) also reported that RBC, HGB, HT and MCHC indices were greater in individuals with BTT than those with IDA. The mean RBC, HGB and HCT were greater in men with IDA than in women, but all seven indices were higher in men than women in BTT group (18).

The present study indicated the best performance based on the AUC belonged to Bor, Ker.I, Ker.II, Sriv and Eh differentiators, and the lowest performance was obtained for Kan.I and Sir Equations. Examination of the value indices of the top 5 differential equations indicated that the highest SEN, NPV and Y.I were seen in Ker.I equation, and the highest SPE, PPV, PLR, NLR and Acc in Ker.II equations. Therefore, the importance of these two indices seems not to be less than Bor (Table 5).

Each of the published articles reported different results depending on the analytical method. Arora et al.(10) selected Men, Matos & Carvalho and Red Cell Distribution Index (RCDI) equations for investigation. Men's equation with a sensitivity of 97.62%, specificity of 66.67%, and Youden's index of 64% was better than the other two indices. However, values of the RCDI differentiator (92.86%, 66.67% and 59%) were a little different from Men. Jahangiri et al. (11) conducted a systematic review of 12 famous differential equations and reported M/H index (ratio of microcytic to hypochromic cells) with sensitivity and specificity of 0.92 and 0.86 respectively. In the discussion of the study, the formula alone could not differentiate BTT from IDA. Kabootarizadeh et al. (6) used a neural network model on blood indices, namely RBC, HGB, MCV and MCH, in Ahvazi couples and reported the sensitivity, specificity and precision of the resulting model equal to 92.33%, 93.13% and 92.5% respectively. The study found the model to be suitable, but it was necessary to test it in

more complex conditions. Jameel et al. (12) studied 135 individuals with microcytic-hypochromic anemia and concluded that the RDWI differential equation (88% sensitivity and 86% specificity) differentiated BTT from IDA better than RDW (80% sensitivity and 86% specificity). The present study also reported the sensitivity and specificity of RDW equal to 91% and 26% respectively. Ullah et al. (15) conducted a cross-sectional study in Pakistan and investigated five differential equations, Men, RDWI, Sh-L, Srivastava and G-K and found a better performance of RDWI with 100% sensitivity and 93% specificity than other equations. In their paper, 570 out of 800 blood samples had IDA and 230 ones had BTT. However, they also discussed the investigation of the issue in larger sample sizes. In a study, among nine famous indices and the innovative differential equation (Sir), the Sir equation and GK and RDWI indices were selected with AUC of 0.914, 0.909, and 0.907 respectively, but there was no significant difference between AUC values of three indices(26). The RDW index had the minimum AUC. Finally, Hashemieh et al. (8) examined the performance of a beta-thalassemia major prevention plan.

Study Limitations

One of the limitations of the study was the small sample size, the other is that despite the advantages and simplicity of the implementation of blood indices, there is a limitation of discriminating formulas since they are not able to differentiate all cases of IDA from BTT.

Conclusion

Although none of the equations could have 100% sensitivity and specificity, five differential equations showed better performance; the Bor equation was in the first place. The equations Ker.I and Ker.II indicates that the Bor index could achieve a greater AUC, but other value indices had maximum amounts in Ker differential equations. Therefore, the importance of two indices seems not to be less than Bor. Previous

studies and their comparison to achieve better differentiation performance and detect their weaknesses indicated that it was necessary to consider the ethnic and racial composition in the population as well as other factors involved in the field as well as test a variety of differentiators and introduce new methods to provide blood and differential indices.

Journalism Ethics considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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Conflict of interest

The authors declare that there is no conflict of interests.

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