Diagnostic reliability check of red cell indices in differentiating Iron Deficiency Anemia (IDA) from Beta Thalassemia Minor (BTT)

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Original Article

Abstract

Introduction: Iron deficiency anemia and beta-thalassemia trait are the two most common causes of hypochromic microcytic anemia. There are some discriminating functions (DFs) formula to differentiate these two conditions based on RBC's indices which would be compared the validity of the 14 various indices in this study.

Methods: A total of 227 patients from 21st March to 21st June 2014 were studied by calculating 14 DFs i.e. Keikhaei (KI), RDWI, Green & King (G & KI), England & Fraser (E & FI), Mentzer (MI), Shine & Lal (S & LI), Telmissani (TI), Ricerca (RI), Ehsani (EI), Keli (RBC) and 4 new indices (F1, F2, F3 & F4). The study included 105 iron deficient patient and 122 beta-thalassemia trait cases (HbA2>3.5 & HbA2<8%). The number of correctly diagnosed cases were determined and sensitivity, specificity, positive and negative predictive value and Youden index of each discrimination index were calculated.

Results: None of the indices showed 100% sensitivity and specificity. G & KI, KI, RDWI, E & FI, F3, EI and MI showed the highest value in differentiating IDA from BTT. The lowest value was belong to S & LI, F4 and F2 indices.

Conclusion: Based on Youden index (YI), the value of discrimination function indices from highest to lowest was as follow: G & KI> KI> RDWI> E & FI> F3> EI> MI> RBC> F1> RI> F2> F4> S & LI.

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Introduction:

Iron Deficiency Anemia (IDA) and Beta thalasemia Trait (BTT) are the most common causes of hypochromic microcytic anemias (1). The incidence of IDA and BTT are 30-45% and 4-10% in Iran, respectively; and estimated there is about 3,750,000 carriers of beta thalassemia (2-4). Because of the high prevalence of IDA and BTT, an easy and simple way to discriminate them is crucial. Determination of Cell Blood Count (CBC) by electronic cell counters is the first step of evaluation of these two conditions (5).

Effective BTT screening could prevent β-thalassemia major births, for which peripheral

blood smear (PBS) (6) and red blood cell indices are invaluable tools for initial diagnosis (7).

Additionally, IDA is an easily curable condition which could be treated by iron replacement supplements. On the other hand, BTT is considered a type of dyerythropoiesis, in which iron deficiency is difficult to exist because of enhanced enteric iron absorption in this condition and hence iron replacement is not effective. However, iron deficiency might coexist or develop in some carriers of beta thalassemia (8,9).

In the last decades, based on red blood cell indices including Hemoglobin (Hb), Hematocrit (Hct), Mean Cell Volume (MCV), RBC count (RBC), Red cell Distribution Width (RDW) and Mean Cell Hemoglobin Concentration (MCHC), many formulas have been proposed to discriminate these two frequent conditions (1). The best known formulae in this regard are England & Fraser (1973); Mentzer (1973); Srivastava & Bevington (1973); Shine & Lal (1977); Green & King (1989); Ricerca et al (1996); Bessman et al.(1999); Jayabose S et al. (1999); Telmissani et al.(1999), Ehsani et al. (2009) and Sirdah et al. (2008) Discriminating Functions (DFs) formulae (5). Based on statistical criteria a valuable test should have a high sensitivity, specificity and Youden Index (YI) (10).

In this study, we compared the validity of various discriminating functions (DFs) formulae in differentiating BTT from IDA by calculating sensitivity, specificity, positive & negative predictive value and Youden's index. Youden's index (YI) takes into account both sensitivity and specificity and gives an appropriate measure of validity of particular technique (10,11).

Methods:

All of the patients with hypochromic (MCH＜27pg) microcytic (MCV＜80 fL in patients older than 6 years and ＜70+age in younger ones) anemia, who were referred to Bandar abbas city's laboratories from 21st March to 21st June 2014, included in this study. Anemia was defined as a hemoglobin concentration of, at least, 2standard deviation lower than age and sex specific average.

For all patients red blood cell indices were obtained by automatic electronic cell counter (Automatic Cell counter: Sysmex-XS800i; Japan), and measurement of serum ferritin level (Liason-chemiluminiscence immunoassay, Diasorin, ferritin kit, Germany), serum iron level with total iron binding capacity (Hitachi 917; Japan, Bionic kits) and Hemoglobin A2 (Capillary electrophoresis: Sebia, France) were done.

A total 244 patients were studied, 120 patients with final diagnosis of IDA and 124 patients with BTT. Patients with acute and chronic inflammation, recent transfusion or bone marrow transplantation and multifactorial anemia cases such as anemia of chronic diseases, other hemoglobinopathies, combined α or β-thalassemia with IDA, and Pregnant women were excluded from study. Regarding to the exclusion criteria, patients with hemoglobin＜9gr/dl or HbA2＞8% were excluded from this study. After exertion of exclusion criteria 227 patients (105 IDA and 122 BTT) were included in the study.

Diagnostic criteria for BTT was considered as HbA2＞3.5% with normal serum ferritin level; and diagnostic criteria for IDA was serum ferritin level ＜12ng/ml, low serum iron level, increased total iron binding capacity and/or a response to iron replacement therapy.

Included patients were grouped based on sex (male & female), age (≤10 y/o & ＞10 y/o), and anemia severity (mild: Hb≥10gr/dl and moderate: Hb＜10 gr/dl).

Finally, 10 common Discriminating Functions indices (DFs) i.e. keikhaei (KI), RDWI, Green&King (G & KI), England & Fraser (E & FI), Mentzer (MI), Shine & Lal (S & LI), Telmissani (TI), Ricerca (RI), Ehsani (EI), Keli (RBC) and 4 new indices (F1,F2,F3 & F4) were calculated using related formulae, according to table 1. Thereafter, calculated indices were interpreted using related cut off points; and finally, sensitivity, specificity, positive and negative predictive value, and Youden index (YI) were calculated for each DFs.

Statistical descriptive assessment and formula making and analysis were done by Excel Office Program 2007, and statistical analysis were done by SPSS 20.

Table 1. Cut-off reference values of different discriminant functions (DFs) to differentiate between IDA and TT (5)

TT	IDA	Formula	DFs
< 21	**≥ 21**	Hb×RDW×100/ (RBC)2×MCHC	KI (Keikhaei) (5)
< 220	**≥ 220**	MCV×RDW/RBC	RDWI (11)
< 65	**≥ 65**	[(MCV)2×RDW]/100×Hb	G&KI (Green & King) (12)
< 0	**≥ 0**	MCV–RBC–(5×Hb)–K*	E&FI (England&Fraser) (13)
< 13	**≥ 13**	MCV/RBC	MI (Mentzer) (14)
<1530	**≥ 1530**	(MCV)2×MCH/100	S&LI (Shine and Lal) (15)
>1.7M, >1.5F	**≤1.7M, ≤1.5F**	MDHL** : MCH×RBC/MCV	TI (Telmissani) (16)
< 3.3	**≥ 3.3**	RDW/RBC	RI (Ricerca) (17)
< 15	**≥ 15**	MCV–10×RBC	EI (Ehsani) (1)
> 5	**≤ 5**	RBC Count	RBC (Keli) (18)
< 2	**≥ 2**	MCV/HCT	F1 (NEW)
< 1.5	**≥ 1.5**	RDW–3 RBC	F2 (NEW)
< 600	**≥ 600**	MCV×RDW–100 RBC	F3 (NEW)
< 10	**≥ 10**	MCV×Hb/RDW×RBC	F4 (NEW)

* K=3.4 (13)
** MDHL: Mean Density of Hb/Liter of Blood.

Results:

A total 227 patients with hypochromic microcytic anemia were entered in this study; including 105 cases with IDA (23 male & 82 female), and 122 cases with BTT (66 male & 56 female); ranging from 1 to 75 years old. Mild anemia was observed in 80% and 85% of patients with IDA and BTT, respectively. Moderate anemia was more frequent in IDA than BTT (20% vs 15%, respectively).

Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (+LR), negative likelihood ratio (-LR), efficacy and Youden's index (YI) of each discriminating function (DF) index is illustrated in Table 2.

Table 2. Diagnostic parameters of 14 discriminative functions

Df	Sensitivity		Specificity		PPV%		NPV%		+LR		-LR		Efficac Y	YI
	IDA	TT	IDA	TT	IDA	TT	IDA	TT	IDA	TT	IDA	TT	IDA/TT	IDA/II
KI	76.19	87.70	87.70	76.19	84.21	81.06	81.06	84.21	6.20	3.68	0.27	0.16	82.38	63.89
RDWI	65.71	91.80	91.80	65.71	87.34	75.68	75.68	87.34	8.02	2.68	0.37	0.12	79.74	57.51
G&KI	74.29	90.16	90.16	74.29	86.67	80.29	80.29	86.67	7.55	3.51	0.29	0.13	82.82	64.45
E&FI	87.62	68.03	68.03	87.62	70.23	86.46	86.46	70.23	2.74	5.49	0.18	0.36	77.09	55.65
MI	70.48	83.61	83.61	70.48	78.72	76.69	76.69	78.72	4.30	2.83	0.35	0.23	77.53	54.09
S&LI	9.52	100	100	9.52	100	56.22	56.22	100	#	1.11	0.90	0.00	58.15	9.52
TI	52.38	86.89	86.89	52.38	77.46	67.95	67.95	77.46	3.99	1.82	0.55	0.25	70.93	39.27
RI	53.33	81.15	81.15	53.33	70.89	66.89	66.89	70.89	2.83	1.74	0.58	0.35	68.28	34.48
EI	72.38	81.97	81.97	72.38	77.55	77.52	77.52	77.55	4.01	2.97	0.34	0.25	77.53	54.35
RBC	65.71	82.79	82.79	65.71	76.67	73.72	73.72	76.67	3.82	2.41	0.41	0.26	74.89	48.50
F1	65.71	81.97	81.97	65.71	75.82	73.53	73.53	75.82	3.64	2.39	0.42	0.27	74.45	47.68
F2	51.43	80.33	80.33	51.43	69.23	65.77	65.77	69.23	2.61	1.65	0.60	0.38	66.96	31.76
F3	62.86	92.62	92.62	62.86	88.00	74.34	74.34	88.00	8.52	2.49	0.40	0.12	78.85	55.48
F4	44.76	81.15	81.15	44.76	67.14	63.06	63.06	67.14	2.37	1.47	0.68	0.42	64.32	25.91

Table 3. Independent sample t-test

	β-T mean (SD)	IDA mean (SD)	t	Sig.(2-tailed)	Mean Difference	Std. Error Difference
Hemoglobin	11.25 (1.38)	10.66 (0.81)	-3.854	0.000	-0.5897	0.1530
Hematocrit	34.45 (4.28)	33.28 (2.21)	-2.520	0.012	-1.1685	0.4637
RBC count	5.55 (0.60)	4.84 (0.47)	-9.860	0.000	-0.71263	0.7228
MCV	62.20 (5.87)	69.24 (6.72)	8.417	0.000	7.033	0.836
MCH	20.30 (1.84)	22.16 (2.33)	6.755	0.000	1.867	0.276
MCHC	32.64 (0.82)	32.00 (1.26)	-4.592	0.000	-0.639	0.139
Keikhaei index	18.69 (2.15)	23.65 (3.97)	11.911	0.000	4.956	0.416
RDWI	186.68 (21.27)	236.16 (39.56)	11.955	0.000	49.478	4.139
Green & King	57.17 (6.70)	73.98 (13.66)	12.019	0.000	16.810	1.399
England & Fraser	-8.01 (7.28)	2.69 (7.08)	11.173	0.000	10.694	0.957
Mentzer index	11.36 (1.88)	14.54 (2.65)	10.508	0.000	3.173	0.302
Shine & Lal	800.06 (231.99)	1092.02 (314.87)	8.021	0.000	291.953	36.400
Telmissani	1.80 (0.25)	1.55 (0.17)	-8.631	0.000	-0.250	0.029
Ricerca index	3.01 (0.34)	3.41 (0.34)	7.259	0.000	0.399	0.055
Ehsani index	6.67 (9.48)	20.83 (10.72)	10.558	0.000	14.159	1.341
Keli index	5.55 (0.60)	4.84 (0.47)	-9.8601	0.000	-0.713	0.072
F1 index	1.82 (0.19)	2.08 (0.20)	10.063	0.000	0.263	0.026
F2 index	-0.21 (1.84)	1.94 (2.34)	7.054	0.000	1.960	0.278
F3 index	472.24 (80.93)	646.65 (154.12)	10.880	0.000	174.410	16.030
F4 index	7.85 (2.09)	9.76 (2.88)	5.792	0.000	1.917	0.331

In this study, overall diagnostic value of DFs in differentiating IDA from BTT was as follow:

G&KI > KI > RDWI > E&FI > F3 > EI > MI > RBC > F1 > TI > RI > F2 > F4 > S&LI

E & FI, KI, G & KI, EI and MI were the most sensitive indices in diagnosis of IDA; and S & LI, F3, RDWI, G & KI and KI were the most sensitive indices in diagnosis of BTT, respectively. For all indices, sensitivity was higher in diagnosis of BTT than IDA, except for E&FI (87.62 vs 68.03 for diagnosis of IDA vs BTT, respectively)

The most valuable DFs in patients younger than 10 y/o was KI (YI=58.37) and G & KI (YI=54.70). On the other hand, G&KI, KI, EI, and MI were the most valuable indices in patient over 6 y/o (YI=69.04, 66.30, 62.31, and 62.25, respectively).

New indices, i.e. F1, F2, F3 & F4, had no more advantages in differentiation between IDA and BTT over previous indices, and mainly had a low diagnostic value, sensitivity and specificity. The only exception was F3 index, with a high sensitivity in diagnosis of BTT (92.62%), specially in men (YI=55.01) with moderate anemia (YI=73.81).

For each parameter, a student t-test was done for equality of means between two groups of IDA and BTT. Differences of means were significant (P-value < 0.001) in all parameters except for HCT (P-value=0.012). Independent sample t-test result are shown in Table 3.

Conclusion:

Iron Deficiency Anemia (IDA) and Beta-thalassemia Minor (BTT) are the most common causes of hypochromic microcytic anemia. Beta thalassemia minor diagnosis is based on microcytosis and elevated HbA2 level (12-14). Iron deficiency anemia diagnostic criteria are decreased serum iron and ferritin level accompanied by increased total iron binding capacity (TIBC) (13-15).

These ways for diagnosis are time consuming and expensive; so discriminative functions (DFs) was suggested as an alternative from 80 decades. The aim of all these indices is to increase the difference of hematologic parameters in IDA and BTT, to make a cut-off point with an acceptable sensitivity and specificity to discriminate these two frequent conditions with hypochromic microcytic anemia. The discriminative power of each index is defined as how much it can amplify the hematological differences between IDA and BTT.

Efficacy of each DF is not only related to the formula itself, but also depends on the hematologic parameters to a larger degree. Based on the differences of hematologic parameters in various populations, each formula could result in different values (5,16-18).

As shown in previous studies, hematologic parameters in different populations greatly depends on various thalassemic gene mutations (1, 19-21); and there is a close relationship between MCV (mean corpuscular volume) and beta-chain mutation in BTT (22). Mild anemia is the other factor which could affect the DF's value. Furthermore, the other disturbing factors, such as population selection bias (children, pregnant women), concurrence of IDA plus BTT and/or alpha thalassemia, and IDA with infection should also be considered in study. It seems all DFs are efficient in differentiation between IDA and BTT, and the critical point which affect the sensitivity and specificity of each DF is the selected cut-off point for each factor (5).

To compare the efficacy of each DF, a valuable statistical index should be selected. A valuable diagnostic test, is the one with a high sensitivity, specificity, efficacy, positive and negative predictive values with a high +LR and low -LR score. For this purpose, Youden's index was selected as a statistical index with the less bias, calculated as follow (10):

Youden index (YI)=(specificity+sensitivity)-100

In this study, the difference of MCV between IDA and BTT groups was less significant than previous studies in iran (5,23), which could be related to a different beta-chain mutation with a less significant affect on MCV in this study.

Furthermore, the sensitivity, specificity and YI of all 14 studied DFs were lower than previous studies. This could be interpreted by the low difference of MCV between IDA and BTT groups, and the great role of this factor in most of studied DFs; which resulted in an increase the role of chance. On the other hand, this could be resulted from the selection of cut-off points from previous studies, which are not optimal for this population.

In this study, the diagnostic value of indices in children were greatly lower than population over 10 years old, as the only indices obtained a YI greater than 50 were KI and G & KI. This could be a sign of population selection bias effect on the relative DFs' values in different populations (5).

The most valuable DFs to differentiate IDA from BTT in this study, based on Youden's index, were G & KI, KI, RDWI, and E & FI. These results were agreed with Keikhaei et.al and disagreed with Ehsani et.al and Rajabiani et.al results which proposed Mentzer, Ehsani and MDHL indices as the most valuable ones. The cause of this similarity and difference could be resulted from difference in selected population and genetic characteristics of the studied regions; as Keikhaei's study was done in south-west of iran, a close geographical situation to our study; while the other 2 studies were performed in north of Iran.

As a conclusion, regarding to the significant differences between sensitivity, specificity and Youden's index of Discriminative functions to differentiate IDA from BTT in this study compared to the others, it's suggested to perform a pilot study to find the best DF and the most optimal cut-off point for each region.

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بررسی ارزش تشخیصی شاخص‌های گلبول قرمز در افتراق آنمی فقرآهن از بتاتالاسمی مینور

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مجله پزشکی هرمزگان سال بیستم شماره سوم ۹۵ صفحات ۱۵۷-۱۵۱

چکیده

مقدمه: *کم‌خونی فقر آهن و خصیصه بتاتالاسمی از شایع‌ترین علل کم‌خونی هیپوکرومیک میکروسیتیک می‌باشند. جهت افتراق این دو بیماری، فرمول‌هایی بر پایه اندکس‌های گلبول قرمز وجود دارد که در این مطالعه به بررسی ارزش این اندکس‌ها پرداخته می‌شود.*

روش کار: *در این مطالعه، جمعاً ۲۲۷ بیمار از اول فروردین تا سی و یکم خرداد ۹۳ مورد بررسی قرار گرفته و اندکس‌های کیخایی، RDWI گرین – کینگ، انگلند – فریزر، منتزر، شایل – ال، تلمیسانی، ریسرکا، احسانی، کلی و چهار اندکس جدید F1 F2 F3 و F4 محاسبه گردید. در این مطالعه، ۱۰۵ بیمار با فقرآهن و ۱۲۲ بیمار با خصیصه بتاتالاسمی (HbA2 > 3.5 & HbA2 < 8%) مورد بررسی قرار گرفتند. تعداد موارد صحیح تشخیص داده شده با استفاده از هر یک از اندکس‌ها ثبت گردید و حساسیت، ویژگی، ارزش اخباری مثبت و منفی بودن هر یک از اندکس‌ها محاسبه گردید.*

نتایج: *هیچ کدام از اندکس‌ها حساسیت و ویژگی ۱۰۰ درصد نداشت. اندکس گرین – کینگ، کیخایی، RDWI انگلند – فریزر، F3 احسانی و منتزر به ترتیب بالاترین ارزش تشخیصی را در افتراق کم خونی فقرآهن از خصیصه بتاتالاسمی را داشتند. پایین‌ترین ارزش تشخیصی مربوط به اندکس‌های شاین – ال، F4 و F2 بود.*

نتیجه‌گیری: *بر پایه اندکس یودن (YI)، ارزش تشخیصی اندکس‌های افتراقی (DFs) به ترتیب زیر می‌باشد:*

G&KI > KI > RDWI > E&FI > F3 > EI > MI > RBC > F1 > TI > RI > F2 > S&LI

کلیدواژه‌ها: *کم خونی هیپوکروم میکروسیتی، کم خونی فقرآهن، خصیصه بتاتالاسمی*

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