IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 14, Issue 7 Ver. I (July. 2015), PP 67-72
www.iosrjournals.org

Hi & Ha, are new indices in differentiation between Iron deficiency anemia and beta-Thalassaemia trait /A Study in Sulaimani City-Kurdistan/Iraq

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Abstract :
Objectives: *This study was conducted to compare the validity of newly created indices, Hisham (Hi index), and Hameed (Ha index) with various discrimination indices in differentiating beta-Thalassaemia trait (β-TT) from iron deficiency anemia (IDA) by calculating their sensitivity, specificity and Youden's index. Methods: in total 600 adult patients with microcytic anemia were involved. New hematological indices (Hi) = (MCH×RDW)/RBC & (Ha) = (MCH×Hct×RDW)/(RBC×Hb)^2 were created, and a total of fifteen discrimination indices; RBCs, RDW, Mentzer index (MI), Shine & Lai index (S&L), England &Fraser index (E&F), Srivastava index (S), Green & King index (G&K), RBC distribution width index (RDWI), Ricerca index (R), Keikhaei index (KI), Telmissani et al index (TI), Ehsani et al index (EI), Sirdah et al index (SI), (Hi) & (Ha) were used to differentiate between these two conditions. Youden's index of each discrimination index was calculated. Results: All fifteen discrimination indices didn't have the sensitivity and specificity of 100%. The Ha & Hi indices showed the most reliable discrimination indices in differentiation between β-TT and IDA. Conclusion: Youden's index of Ha & Hi was the highest and most reliable in differentiating β-TT from IDA in the adult patient.*
Keywords - *ß-Thalassaemia trait, discrimination indices, indices, iron deficiency anemia , red blood cell*

I. Introduction

The high-performance liquid chromatography (HPLC) is considered a standard method of screening for β-TT, but it is costly and not available routinely. Screening by cell counter–based parameters and formulas is rapid, automated, inexpensive, and technically sound. At present, cell counters are widely used in routine practice, so screening can be done without additional costs to medical systems. [(1,2)] To date many discrimination indices have been reported using red blood cell indices obtained by automated blood count. Many authors calculated sensitivity and specificity of these discrimination indices in distinction between IDA and β-TT. [(3-6)]. However, none of these indices has a sensitivity and specificity of 100% in prediction of IDA and β-TT. Some of them have a considerable sensitivity for IDA or β-TT but not specificity. [(6, 7)] Youden's index provides an appropriate measure of validity of a particular technique or question by taking into account both sensitivity and specificity. [(8, 9)] The performance of each discrimination indices does not only depend on the formula itself, but is also related mostly to hematological parameters. Based on the hematological parameter differences among populations studied, the varieties of formula results may be observed. [(10- 12)]

II. Method and statistics

In total, 600 adult patients with microcytic anemia were involved in this study. New hematological indices were created; Hisham index (Hi) = (MCH×RDW)/RBC, and Hameed index (Ha) = (MCH×Hct×RDW)/ (RBC×Hb)^2. These new indices in addition to several other discrimination indices have been proposed to distinguish between IDA and β-TT. In the present study fifteen discrimination indices were calculated; RBCs, RDW, Mentzer index (MI), Shine & Lai index (S&L), England &Fraser index (E&F), Srivastava index (S), Green & King index (G&K), RBC distribution width index (RDWI), Ricerca index (R), Keikhaei index (KI), Telmissani et al index (TI), Ehsani et al index (EI), Sirdah et al index (SI), Hisham index (Hi) & Hameed index (Ha). In this study 228 patients with IDA and in 372 patients with β-TT were confirmed by HPLC, SI & TIBC test. The number of correctly identified patients was determined by using each discrimination index. The sensitivity, specificity, positive and negative predictive value and Youden's index of each discrimination index were calculated.

The differential values for each discrimination index were applied as defined in the original published reports. Complete blood counts were obtained by Beckman coulter Hmx & Horiba ABX micros 60System. Serum iron and SIBC were determined calorimetrically and ferritin was measured by radioimmunoassay. The values of HbA2 were determined by Bio-Rad Laboratories, California/USA. Utilizes the principles of cation-

exchange high-performance liquid chromatography (HPLC).The sensitivity and specificity, and positive and negative predictive values were calculated as follows: sensitivity: true positive/(true positive + false negative); specificity: true negative/(true negative + false positive); positive predictive value: true positive/(true positive + false positive); negative predictive value: (true negative/true negative + false negative); Youden's index is calculated as (sensitivity + specificity) – 100.

III. Result

The most frequently encountered diseases with mild microcytic anemia are β-TT and IDA. In addition to genetic counseling for identification of Thalassaemia carriers in order to prevent the birth of Thalassaemia patients, differentiating β-TT from IDA is warranted because the Thalassaemia minor should not be given iron in attempt to normalize MCV. The diagnosis of β-TT is established by the presence of RBC microcytosis and elevated levels of HbA2 [(13)]

Decreased levels of serum iron and ferritin with increased levels of SIBC are the main diagnostic criteria for IDA. [(14)] Less time-consuming methods are based on the calculation of many discrimination indices from blood parameters obtained during routine complete blood count. These indices incorporate MCV, MCH, RBC count, RDW, Hct and Hb in various combinations. For a certain degree of anemia, RBC tend to be more microcytic and hypochromic in β-TT than in iron deficiency states. As a result, MCV and MCH tend to be lower in β-TT compared to IDA. On the contrary, RBC count tends to be higher in β-TT than in IDA. Thus, most indices use MCV, MCH, and RBC count to amplify these differences. Most of them are based on the fact that, in iron deficiency anemia, the anisocytosis is more predominant than in beta thalassemia trait. Microcytosis is usually more predominant in beta thalassemia trait than in iron deficiency and it is proportional to the degree of anemia in iron deficiency.

According to the original published papers by the authors of other indices, their sensitivity in the detection of β-TT and IDA is approximately 100%. However, later studies failed to confirm these results and estimate these indices' sensitivity between 61–91 %. [(15)] In the present study the sensitivity was ranged between 25%-97% and that support the fact, that there is no such index with full sensitivity for this aim. As each index showed overlapping values in patients with β-TT and IDA, none of them was entirely satisfactory in discriminating between these conditions.

All the studies show that the RBC count is the best discriminative hematological parameter in differentiation of β-TT and IDA[(11-15)]. In the present study and as shown in Table 3.1, the mean of RBC was found (4.56×10^{12}/L) in IDA cases and (5.9×10^{12}/L) in β-TT cases and as expected this is in agreement with others. From the15 discrimination blood indices including the two newly created indices; Ha & Hi as shown in the Table 3.2 are the highest in correct identification of the patients. These two indices also had the highest Youden's index value (88%) and (87%) respectively as in Table3.3. Majority of researchers work in this issue considered that RBC and RDW are the highest indices according to the Youden's index [(6,11,12,15)], but this is not confirmed by the present study. A local variation in blood indices or the MCH used in both newly created indices probably has a role in these differences which may also explain the difference in the consequences of other indices according to the Youden's index from others. Table 3.3 shows that sensitivity of both Ha and Hi indices are not the highest but, they are competitive to the others. [(11,12,15)]. Our data showed that the new indices (Ha & Hi) could be used as a sufficient tools for differentiating between these two disorders. Youden's index takes into account both sensitivity and specificity and gives an appropriate measure of validity of a particular question or technique. As physicians, how many of us memorize these formulas and use them in our daily practice in crowded outpatient settings? However, we do consider these indices, which are easy to calculate.

How many of us would be brave enough to ignore the study Hb- electrophoresis in a woman with mild hypochromic anemia unresponsive to iron therapy who was planning a pregnancy? However, in large number of patients, it would enough to do one of the tests which are required to confirm the diagnosis β-TT or IDA, especially with these new indices and that will reduce the cost and effort. The examination of the blood film and the applying these indices for differentiation between β-TT and IDA will be enough in majority of cases without doing confirmatory tests, especially in the regions are deficient in confirmatory tests.

As shown in the table 3.4 which includes 600 cases, we are able to completely diagnose some patients of β-TT and IDA that are located upper and lower the overlapping area; in Hameed index we are able to diagnose 46% of IDA, 58% of β-TT, totally 53.5% for both types without doing any confirmatory tests (100% positive). In Hisham index 35.5% of IDA, 55% of β-TT, totally 47.5% for both, while in RBC count 6% of IDA, 27% of β-

TT, totally 19% for both. So depending on Hameed index we are able to decide completely for more than half of patients in microcytic cases wither it is β-TT or IDA.

IV. Tables

Table 2.1 Threshold values of the indices used in this study.		
Indices	**IDA**	**B-TT**
Red blood cell count (RBC)	< 5	> 5
RBC distribution width(RDW)	>16	<16
Mentzer index (MI) = MCV/RBC	> 13	< 13
Shine and Lal (S&L) index = MCV2 × MCH × 0.01	> 1530	< 1530
England and Fraser (E&F) index = MCV – RBC – 5Hb – 8.4	>0	<0
Srivastava index (S) = MCH/RBC	> 3.8	< 3.8
Green and King (G&K) index = MCV2 × RDW/100Hb	> 65	< 65
RBC distribution width index (RDWI) = MCV × RDW/RBC	> 220	< 220
Ricerca (R) index = RDW/RBC	> 3.3	< 3.3
Keikhaei index (KI) = Hb×RDW×100/ (RBC)2×MCHC	>21	<21
Telmissani et al index (TI) MCHD: MCH/MCV MDHL: MCHD ×RBC	<1.7M <1.5F <1.6	>1.7M >1.5F >1.6
Ehsani et al index (EI) = MCV–10×RBC	>15	<15
Sirdah et al index (SI) = MCV–RBC–3×Hb	>27	<27
Hisham index (Hi) = (MCH×RDW)/RBC	≥67	< 67
Hameed index (Ha) =(MCH×Hct×RDW)/(RBC×Hb)^2	≥220	< 220
MCV, mean cell volume; MCH, mean corpuscular hemoglobin; Hb, hemoglobin. MCHD: Mean Cell Hb Density, MDHL: Mean Density of Hb/Liter of Blood.		

Table 3.1 Hematological data of study groups				
	IDA n=228		**β-TT n=372**	
Hematological data	Range	Mean [± SD]	Range	Mean [± SD]
Hb (g/dl)	**6.7-14.8**	**10.5 (**± 1.3**)**	**8-16**	**12.1 (**± 1.3**)**
RBC (×10^{12}/L)	**3.25-5.88**	**4.56 (**± 0.42**)**	**4.06-7.65**	**5.9 (**± 0.6**)**
Hct (%)	**21.2-46.6**	**33.2 (**± 3.8**)**	**25.2-50.9**	**39 (**± 4.3**)**
MCV (fl)	**55.5-87.7**	**73 (**± 6.8**)**	**52.2-84**	**66 (**± 4.8**)**
MCH (pg)	**16.6-35.9**	**23.2 (**± 2.7**)**	**16.1-26.4**	**20.5(**± 1.8**)**
MCHC (g/dL)	**27.9-34.2**	**31.7 (**± 1.1**)**	**27.4-35.6**	**31.1(**± 1**)**
RDW (%)	**11.9-23.1**	**16.4 (**± 2**)**	**12.1-20**	**15(**± 1.2**)**
β-TT, beta thalasemia 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.				

Table 3.2
The differential values of each discrimination index and correctly identified number of patients

Differential values		IDA (n=228)	β□-TT (n=372)	Total correctly identified patients (n=600)	Percentage of correctly identified patients (%)
RBC	IDA< 5 β-TT>5	+202 -26	-21 +351	553(202+351)	92.2%
RDW	IDA >16 β-TT<16	+193 -35	-61 +311	504(193+311)	84
MI	IDA >13 β-TT<13	+210 -18	-54 +318	528(210+318)	88
S&L	IDA >1530 β-TT<1530	+57 -171	-11 +361	418(57+361)	70
E&F	IDA >0 β-TT<0	+205 -23	-36 +336	541(205+336)	90.2
S	IDA >3.8 β-TT<3.8	+216 -12	-96 +276	492(216+276)	82
G&K	IDA >65 β-TT<65	+213 -15	-31 +341	554(213+341)	92.3
RDWI	IDA>22o β-TT<220	+202 -26	-10 +362	564(202+362)	94
R	IDA >3.3 β-TT<3.3	+171 -57	-11 +361	532(171+361)	88.7
KI	IDA >21 β-TT<21	+209 -19	-22 +350	559(209+350)	93.2
TI	IDA <1.6 β-TT>1.6	+200 -28	-39 +333	533(200+333)	89
EI	IDA >15 β-TT<15	+211 -17	-49 +323	534(211+323)	89
SI	IDA >27 β-TT<27	+219 -9	-87 +285	504(219+285)	84
Hi	IDA >67 β-TT<67	+210 -18	-17 +355	565(210+355)	94.2
Ha	IDA >220 β-TT<220	+207 -21	-11 +361	568(207+361)	94.7

+True positives;- True negative; β-TT: Beta thalassemia trait; IDA: Iron deficiency anemia; RBC: Red blood cells; RDW: Red blood cell distribution width; MI: Mentzer index; S & L: Shine and Lal index; E & F: England and Fraser index; S: Srivastave index; G & K: Green and King index; RDWI: Red blood cell distribution width index; R: Ricerca index; KI: Keikhaei index; TI: Telmissani et al index; EI: Ehsani et al index; SI: Sirdah et al index; Hi: Hisham index; Ha: Hameed index.

Table-3.3
Diagnostic parameters of 15 discriminative functions for patients with IDA and patients with the β-TT

Index		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Efficiency (%)	Youdens Indices
Red blood cell (RBC)	IDA β-TT	88.6 94.3	94.3 88.6	90.5 93	93 90.5	92	83
RBC distribution width (RDW)	IDA β-TT	85 84	84 85	76 90	90 76	84	69
Mentzer index (MI)	IDA β-TT	92.1 85.5	85.5 92.1	79.5 94.6	94.6 79.5	88	77.5
Shine & Lal (S&L) index	IDA β-TT	25 97	97 25	84 68	68 84	70	22
England &Fraser (E&F) index	IDA β-TT	90 90.3	90.3 90	85 93.5	93.5 85	90.2	80
Srivastava index (S)	IDA β-TT	94.7 74.2	74.2 94.7	69 96	96 69	82	69
Green & King (G&K) index	IDA β-TT	93.4 91.7	91.7 93.4	87 96	96 87	92.3	85
RBC distribution width index (RDWI)	IDA β-TT	88.6 97.3	97.3 88.6	95.3 93.3	93.3 93.3	94	86
Ricerca (R) index	IDA β-TT	75 97	97 75	94 86.4	86.4 94	88.7	72
Keikhaei index (KI)	IDA β-TT	91.7 94	94 91.7	90 95	95 90	93.2	85.7
Telmissani et al index (TI) (MDHL)	IDA β-TT	88 89.5	89.5 88	84 92	92 84	89	77.5
Ehsani et al index (EI)	IDA β-TT	92.5 87	87 92.5	81 95	95 81	89	79.5
Sirdah et al index (SI)	IDA β-TT	96 77	77 96	71.5 97	97 71.5	84	73
Hisham index (Hi)	IDA β-TT	92.1 95.4	95.4 92.1	92.5 95	95 92.5	94.2	87
Hameed index (Ha)	IDA β-TT	91 97	97 91	95 94.5	94.5 95	94.7	88

Sensitivity = true positive/(true positive + false negative); specificity = true negative/(true negative + false positive); positive predictive value (PPV) = true positive/(true positive + false positive); negative predictive value (NPV) = true negative/(true negative + false negative); Youden's index = (sensitivity + specificity) – 100; Efficacy = (true positive + true negative)/(true positive+ true negative + false positive + false negative).

Table 3.4 Overlapping of some Hematological data & new indices depending on this study.

	IDA n=228			β-TT n=372			IDA &β-TT N=600
Hematological data & new indices	Over lapping	100% positive	Percentage of 100% positive	Over lapping	100% positive	Percentage of 100% positive	Percentage of100% positive
Ha	> 268	105	46%	< 170	216	58%	53.5%
Hi	> 87	81	35.5%	< 52.5	204	55%	47.5%
RBC ($\times 10^{12}$/L)	< 406	14	6%	> 5.88	101	27%	19%
Hct (%)	< 25	10	4.4%	> 46	15	4%	4%
Hb (g/dl)	< 8	8	3.5%	> 14.8	5	1.3%	2.2%

β-TT, beta thalassemia trait; Hb, hemoglobin; IDA, iron deficiency anemia; RBC, Red blood cells; RDW, Red blood cell distribution; Hct, Haematocrit; Hi, Hisham index & Ha, Hameed index.

V. Conclusion

The new indices (Ha & Hi) could be used as a sufficient tools for differentiating between β-TT and IDA, to decrease the cost of the tests, especially in low economic areas; physicians can use one of them.

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