Assessment of new indices to discriminate between iron deficiency anemia and thalassemia trait in Sohag University

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Abstract

Background: Beta-thalassemia trait and iron deficiency anemia are among the most common types of microcytic anemia encountered by pediatricians. Distinguishing β-TT from IDA has important clinical implications because each disease has an entirely different cause, prognosis, and treatment. Misdiagnosis of β-TT has consequences for potential homozygous offspring. Objective: The major purpose of this study is to evaluate the validity of 23 new indices to discriminate between iron deficiency anemia and thalassemia trait in developing countries to decrease the cost and time of the screening programs. Methodology: This study included 200 patients who presented with microcytic hypochromic anemia by CBC at the pediatric hematological outpatient clinic of Sohag University Hospital and their ages ranging from 6 months to 16 years from May 2017 to May 2018. Results: The results showed that Kerman Index 1 had the highest Youden's Index value (61.02%) in rightly differentiating between IDA and B-TT, while it was found that Sirachainan et al, Huber-Herklotz Index (HH), and RDW as a discriminative function showed the worst Youden index (0.0%, -1.69%, and -5.65% respectively) and ineffective in differentiating between microcytic anemias in our population. Conclusion: The present study indicates that Kerman Index 1, Mentzer index (MI), Ehsani et al index, Modified Mentzer index, Kerman Index 2, and RBCs count are the highest reliable discriminator indices in differentiating of β-TT and IDA in Sohag country.

Keywords: IDA, β-TT, RBC

Introduction

Disorders interfering with the formation or rate of production of hemoglobin (Hb) can induce a reduction in mean red cell Hb and corpuscular volume (MCV) with resultant hypochromia and microcytosis [1, 2].

We all know that the commonest single nutrient disease in human beings is iron deficiency anemia (IDA) which causes diseases in nearly 1 billion people all over the world [3].

The most widespread genetic disease in the world is considered to be β-thalassemia. Between 1.5 and 7%, 0f the population all over the world carries one of the genes that cause disorder in hemoglobin formation, and nearly 60,000 a year are diagnosed as β-thalassemia patients [4]. Carriers 0f β-thalassemia are usually clinically asymptomatic [5].

A full and complete clinical history is essential to make the correct and accurate diagnosis. Determinations of red blood corpuscular (RBC) indices by electronic cell counters represent the first step for the mass screening in the population for β-TT. Measurement of the serum ferritin level (<10 ng/ml) in screened subjects with hypochrom­ia and/0r microcytosis is the next second step to exclude IDA cases. The next step is the diagnosis 0f β-TT by quantitation 0f HbA2 level (>3.5%) [2, 6].

Several researchers have tried to use red blood cells count parameters for the recognition of suspected β-thalassemia carriers put side by side to patients with IDA or other normal individuals by determining cutoff values [7].

The first attempts to use mathematical formulations were made in the early 1970s [8, 9].

Although, no one of these indices pointed out the sensitivity and specificity of 100% in the prediction of IDA and B-TT. Many of them viewed significant sensitivity for IDA or for TT but didn't show valuable specificity. The diagnostic reliability of these discriminant approaches is controversial [10]. Y0uden's index provides an appropriate measure of the validity of a particular technique or question by taking into consideration both sensitivity and specificity [11].

The varieties of results of the formula may be detected, due to the hematological parameter differences between the patients of the study [12].

Aim of the work

The major purpose of this study is to evaluate the validity of 23 new indices to discriminate between iron deficiency anemia and thalassemia trait in developing countries to decrease the cost and time of the screening programs.

Patients and Methods

Place of the study: Pediatric Hematological Outpatient Clinic.

Type of the Study: a prospective hospital-based study.

Study Period: one year.

Study population: This study included 200 patients who presented with microcytic hypochromic anemia by CBC at Pediatric Hematological Outpatient Clinic of Sohag University Hospital and their ages ranged from 6

months to 16 years in the period from May 2017 to May 2018.

Patients:

Inclusion Criteria:

- All patients with microcytic hypochromic anemia aged from 6 months to 16 years were enrolled in this study.
- Anemia defined as Hb levels between 8.7–11.4 gram/deciliter.
- Microcytosis defined as MCV < 80 FL at age more than 6 years or MCV < 70 FL at age less than 6 years.
- Hypochromia defined as MCH< 30 g/dL at age < 2 years and < 31g/dL at any other age.

Exclusion Criteria:

- All patients with obvious symptoms of infectious diseases or acute or chronic inflammation or had received a transfusion or had an acute bleeding attack in the last month.
- All patients with Hb levels <8.7 g/dL as these cases of severe anemia are not mistaken as beta-thalassemia trait in our daily practice

Ethical consideration:

Oral and written consent was taken from parents of patients included in the study and were introduced to the ethics scientific committee at Sohag University Hospital for approval.

Methods of the study:

All patients in this study were subjected to the following investigation:

Fasting venous blood samples were obtained after the children had a 30-minute rest in a sitting position. Two blood samples were taken from each child and used for complete blood count (CBC) test and HbA2 analysis. The other one was collected and then centrifuged for 5 minutes to get the serum. The serum was used for the SI, SF, total iron-binding capacity.

The differential indices used in the evaluation were assessed and used to differentiate between β-TT and IDA.

The Sensitivity, the specificity, the positive predictive value (PPV), the

negative predictive value (NPV), &Youden's index were computed for each and all are calculated as follows:

- Sensitivity (Se) means true positive/ (true positive + false negative).
- Specificity (Sp) means true negative/ (true negative + false positive).
- Positive-predictive value (PPV) means true positive/ (true positive + false positive).
- Negative-predictive value (NPV) means true negative/ (true negative + false negative).
- YI was calculated as (Se + Sp) – 100.

Receiver operative characteristic (ROC) curves were constructed to calculate the area under the curve (AUC) for each index to establish new cut-off values.

When the AUC of the parameter is near to 1, the revised cut-off could be considered.

The discrimination indices were tested with old and revised cut-offs for each case.

Statistical analysis:
Data were analyzed using SPSS version 17 and Medcalc version 15.8.0. Quantitative data were expressed as means ± standard deviation, median, and range.

Qualitative data were expressed as number and percentage.

Quantitative data were tested for normality by the Shapiro–Wilk test.

Mann–Whitney U test was used for data that wasn't normally distributed.

Chi-square (χ2) test was used for comparison of qualitative variables.

Receiver operating characteristic (ROC) curve was constructed, for optimum cut off point of the studied indices in predicting thalassemia trait, and the area under the ROC curve value with 95% CI was calculated. The optimal cut-off value was determined; sensitivity, specificity, positive predictive value, negative predictive value, and Youden index were calculated.

A 5% level was chosen as a level of significance in all statistical tests used in the study.

Results

This was a prospective hospital-based study that was conducted on 200 children & adolescents (75 females & 125 males) who were presented to the Pediatric Outpatient Clinic of Hematology Unit at Sohag University Hospital with microcytic hypochromic anemia diagnosed by CBC from May 2017 to May 2018.

One hundred and seventy-seven (88.5%) patients were diagnosed with IDA and twenty-three (11.5%) patients had β-TT by hemoglobin electrophoresis.

The age of cases ranged from 6 months to 16 years with mean age was 2.73 ± 2.53 with male predominant (65%) in IDA; and 7.87± 4.93 with female predominant (56.5%) in β-TT and weight ranged from 6 kg to 37kg with mean weight 12.47 ± 5.48 for IDA & 24.83 ± 10.52 for β -TT. Table **(1).**

Age (years) Mean± S.D.	**2.73 ± 2.53**	**7.87± 4.93**	**<0.001**
Median (Range)	**1.7 (0.5 – 12)**	**9 (3 – 16)**	
Gender Female	**62 (35%)**	**13 (56.5%)**	**0.045***
Male	**115 (65%)**	**10 (43.5%)**	
Weight (Kg): Mean± S.D.	**12.47 ± 5.48**	**24.83 ± 10.52**	**<0.001**
Median (Range)	**10.5 (6 – 34)**	**29 (12 – 37)**	

Table (1): Comparison between the study groups regarding age, gender, and weight.
P-value is calculated by Mann-Whitney U test * P-value is calculated by Chi-Square test, P-value <0.05 is statistically significant

Comparison between the study groups regarding laboratory investigation was found in Table 2 and show that:

The mean corpuscular volume (MCV) in IDA group was 63.24 ± 6.86 FL and this was higher than that in the β-TT group which was 58.16± 2.87 FL, so there was a statistically significant difference between the two groups (p-value <0.001).

The mean corpuscular hemoglobin (MCH) in IDA group was 20.61± 3.59 pg./dL which was higher than that in the β-TT group that was 18.36 ± 0 .77 pg./dL, so there was a statistically significant difference between the two groups (p-value 0.002).

The mean corpuscular hemoglobin concentration (MCHC) in IDA group was 31.83 ± 2.84 g/dL, which was higher than that in the β-TT group that was 30.97 ± 0.79 g/dL, so there was a statistically significant difference between the two groups (p-value 0.005).

laboratory investigation	Iron deficiency anemia group (N= 177)	Thalassemia trait group (N= 23)	P-value
MCV (fl.) Mean± S.D. Median (Range)	63.24 ± 6.86 66.2 (48.7 – 80.5)	58.16± 2.87 57 (55 – 63.5)	<0.001*
MCH (pg.) Mean± S.D. Median (Range)	20.61± 3.59 21 (13.5 – 27.5)	18.36± ٠ .77 18 (16.8 – 19.6)	٠.002*
MCHC (g/dl) Mean± S.D. Median (Range)	31.83± 2.84 32.1 (20.4 – 36.4)	30.97 ± ٠.79 31 (30 – 32)	٠.00٥*
HCT (%) Mean± S.D. Median (Range)	29.64 ± 3.84 29.4 (22.9 – 38)	31.56 ± ٠.48 31.7 (31 – 32.1)	0.00٤*
Hb (g/dl) Mean± S.D. Median (Range)	9.63 ± ٠.85 9.5 (8.65 – 11.4)	9.67 ± ٠.29 9.7 (9.2 – 10)	٠.332
RDW (%) Mean± S.D. Median (Range)	20.05 ± 4.12 18.8 (13 – 28.1)	19.5 ± 3.42 17.5 (16.5 – 25.1)	٠.431
RBCs count (millions/cmm) Mean± S.D. Median (Range)	4.62 ± ٠.65 4.56 (3.37–6.4)	5.37± ٠.38 5.5 (4.63 – 5.79)	<0.001*
Serum iron (ug/dl) Mean± S.D. Median (Range)	39.05± 28.18 35 (8 – 118.7)	67.83 ± 29.85 48 (46 – 120)	<0.001*
Serum ferritin (ng/ml) Mean± S.D. Median (Range)	48.04 ± 66.48 20 (2.5 – 318.5)	29.41 ± 10.66 43.1 (10.52 – 40)	٠.418
Total iron-binding capacity (ug/dl) Mean± S.D. Median (Range)	386.14± 67.76 399 (228.6– 530)	288.13 ± 24.96 300 (250 –318)	<0.001*

Table (2): Comparison between the study groups regarding laboratory investigation

P-value is calculated by Mann-Whitney U test.

*P-value <0.05 is statistically significant.

RBC, red blood cells; Hb, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW, red blood cell distribution width.

The mean red blood cell distribution width (RDW) in IDA group was 20.05 ± 4.12 % which was similar to that in the β-TT group that was 19.5 ± 3.42 %, so there was no statistically significant difference between the two groups (p-value 0.431).

We found that the mean of serum iron (SI) in IDA group was 39.05 ± 28.18 ug/dL and this was lower than that in the β-TT group which was 67.83 ± 29.85 ug/dL, so there was a statistically significant difference between the two groups (p-value <0.001).

The mean serum ferritin level in IDA group was 48.04 ± 66.48 ng/ml ranging from 2.5 to 318.5 ng/ml while in the β-TT group it was 29.41 ± 10.66 ng/ml ranging from $10.52 - 40$ ng/ml, so there was no statistically significant difference between the two groups (p-value 0.418).

The mean of total iron-binding capacity in IDA group was 386.14 ± 67.76 ug/dl which was higher than that in the β-TT group which was 288.13 ± 24.96 ug/dl, so there was a statistically significant difference between the two groups (p-value <0.001).

Table (3): sensitivity, specificity, negative predictive value, positive predictive value, and Youden's index of the studied indices using the published cutoff point.

Marker	Old published Cut off	IDA (n=177)	B-TT (n=23)	Sensitivity (%)	Specificity (%)	YI (%)	PPV (%)	NPV (%)
Mentzer index (MI) IDA β-TT	>13 <13	104 73	0 23	100.00	58.76	58.76	23.95	100
Modified Mentzer index (MI) IDA β-TT	>0 <0	104 73	0 23	100.00	58.19	58.19	23.95	100
RDWI IDA β-TT	>220 <220	141 36	8 15	65.22	79.66	44.88	29.41	94.63
Shine and Lal index IDA β-TT	>1530 <1530	2 175	0 23	100	1.13	1.13	11.61	100
Srivastava and Bevington index IDA β-TT	>3.8 <3.8	120 57	4 19	82.61	67.80	50.41	25	96.77
Green and King index IDA β-TT	>65 <65	128 49	9 14	60.87	72.32	33.19	22.22	93.43
Sirdah index IDA β-TT	>27 <27	117 60	6 17	73.91	66.10	40.01	22.07	95.12
Ehsani index IDA β-TT	>15 <15	104 73	0 23	100	58.76	58.76	23.95	100
England and Fraser index IDA β-TT	>0 <0	148 29	14 9	39.13	83.05	22.18	23.68	91.35

	cut-off							
Ricerca index IDA β-TT	>3.3 <3.3	146 31	9 14	60.87	82.49	43.36	31.11	94.19
MDHL (male) IDA β-TT	<1.7M,<1.5F >1.7M,>1.5F	97 18	5 5	50	84.35	34.35	21.73	95.09
MDHL (female) IDA β-TT	<0.3045 >0.3045	28 34	0 13	92.31	43.55	35.86	27.65	100
MCHD IDA β-TT	>21 <21	39 138	6 17	26.09	77.97	4.06	10.96	86.66
Keikhaei index IDA β-TT	<14 >14	142 35	9 14	60.87	80.23	41.1	28.57	94.03
Sirachainan index IDA β-TT	<44.76 >44.76	177 0	23 0	0	100	0	-	88.5
Bordbar index IDA β-TT	>.3095 <0.3095	46 131	0 23	100	25.99	25.99	14.93	100
Differentiating score for male IDA β-TT	>23 <21	65 50	5 5	50	56.52	6.52	9.090	92.85
Differentiating score for female IDA β-TT	321-370 <250	42 20	4 9	69.23	67.74	36.97	31.03	91.30
Huber-Herklotz Index (HH) IDA β-TT	10.5-13 <8	174 3	23 0	0	98.31	-1.69	0	88.32
Kerman Index 1 IDA β-TT	>67 <67	34 69	0 23	100	61.02	61.02	25	100
Kerman Index 2 IDA β-TT	>220 <220	37 69	0 23	100	65.09	56.67	25	100
Hisham Index (Hi) IDA β-TT	>14 <14	134 43	9 14	60.87	75.71	36.58	24.56	93.70
Hameed Index (Ha) IDA β-TT	<5 >5	0 177	0 23	100	0	1	11.5	-
RDW IDA β-TT	>13 <13	168 9	23 0	0	94.35	-5.65	0	87.95
RBCs count IDA β-TT	>0 <0	126 51	4 19	82.61	71.19	53.8	27.14	96.92

RBC, red blood cells; RDW, red blood cell distribution width; MCHD, mean cell Hb density; MDHL, mean density of Hb/liter of blood.

(cut-off values transformed into generally used units: Hb in g/dL; RBC in 1012/L; MCV in FL; MCH in pg.; HCT in % MCHC in g/dL; RDW in %).

The 23 investigated discriminant functions were applied in all cases in this study and show the following:

As regards the old cut-off values available before:

Kerman Index 1, Mentzer index (MI), Ehsani et al index, Modified Mentzer index, Bordbar et al, Shine and Lal, Hameed Index, and Kerman Index 2 show the best **sensitivity** (100%) followed by MDHL which show sensitivity (92.31%) then all other indices from the highest to lowest was RBCs count (82.61%), Srivastava and Bevington index, (82.61%) Sirdah et al index, (73.91%)Differentiating score for female, (69.23%) RDWI, (65.22%), Ricerca et al index (60.87%), Keikhaei index (60.87%), Hisham Index (Hi) (60.87%), Green and King index (60.87%), MDHL (50%), Differentiating score for male (50%), England and Fraser index (39.13%), MCHD (26.09%), while Sirachainan et al, Huber-Herklotz Index (HH), and RDW wear not sensitive at all there sensitivity were (0.0%).

On the other hand the highest **specificity** belong to Sirachainan et al (100%) then Huber-Herklotz Index (HH) (98.31%) followed by RDW (94.35%) and all other indices from highest to lowest was MDHL for male (84.35%), E & F index (83.05%),

Ricerca et al index (43.36%), Keikhaei index (41.1%), Sirdah et al index (40.01%), Differentiating score for female (36.97%), Hisham Index (Hi) (36.58%), MDHL (35.86%), MDHL (34.35%), Green and King index (33.19%), Bordbar et al (25.99%), and England and Fraser index (22.18%). Differentiating score for male, MCHD, Shine, and Lal, and Hameed Index show the lowest Youden index (6.52%,4.06%,1.13%, and 1%, respectively).

While Sirachainan et al, Huber-Herklotz Index (HH), and RDW show

Ricerca et al index (82.49%), Keikhaei index (80.23%), RDWI (79.66%), MCHD (77.97%), Hisham Index (Hi) (75.71%), Green and King index (72.32%),,RBCs count (71.19%), Srivastava and Bevington index (67.8%), Differentiating score for female (67.74%), Sirdah et al index (66.1%), Kerman Index 2 (65.09%), Kerman Index 1 (61.02%), Mentzer index (MI)(58.76%), Ehsani et al index (58.76%), Modified Mentzer index (58.19%), Differentiating score for male (56.52%), MDHL for female (43.55%), Bordbar et al (25.99%), the two lowest indices in specificity were Shine and Lal (1.13%) and Hameed Index (0.0%) which has no specificity at all.

As regard **Youden index,** that takes into account both sensitivity and specificity and gives an appropriate measure of the validity of a particular technique**,** the highest index was Kerman Index 1 (61.02%) which could be a reliable discriminator between β-TT and IDA, followed by Mentzer index (MI) (58.76%),and then the rest of indices from highest to lowest Ehsani et al index (58.76%), Modified Mentzer index (58.19%), Kerman Index 2 (56.67%), RBCs count (53.8%), Srivastava and Bevington index (50.41%), RDWI (44.88%), the worst Youden index (0.0%, -1.69%, and -5.65% respectively).

Ricerca et al index shows the best **Positive predictive value** equal (31.11%), followed by Differentiating score for female (31.03%), then other indices arranged from highest to lowest as follow:

RDWI (29.41%), Keikhaei index (28.57%), MDHL (27.65%), RBCs count (27.14%), Srivastava and Bevington index (25%), Hisham Index (Hi) (24.56%), Kerman Index 1 (24.17%), Kerman Index 2 (24.17%), Mentzer index (MI) (23.95%), Ehsani et al index (23.95%), Modified

Mentzer index (23.95%), England and Fraser index (23.68%), Green and King index (22.22%), Sirdah et al index (22.07%), MDHL (21.73%), Bordbar et al (14.93%), Shine and Lal (11.61%), Hameed Index (11.5%), MCHD (10.96%), and Differentiating score for male (9.09%).

Huber-Herklotz Index (HH), and RDW show the lowest PPV which were (0.0%) for both.

PPV for Sirachainan et al index can't be calculated.

MDHL, Kerman Index 1, Kerman Index 2 Mentzer index (MI), Ehsani et al index, Modified Mentzer index, Bordbar et al, Shine and Lal all show the best **Negative predictive value** which was 100% for all of them followed by other indices that arranged from highest to lowest as follow: RBCs count (96.92308%), Srivastava and Bevington index (96.77419%), Sirdah et al index (95.12195%), MDHL (95.09804%), RDWI (94.63087%), Ricerca et al index (94.19355%), Keikhaei index (94.03974%), Hisham Index (Hi) (93.70629%), Green and King index (93.43066%), Differentiating score for male (92.85714%), England and Fraser index (91.35802%), Differentiating score for female (91.30435%), Sirachainan et al (88.5%), Huber-Herklotz Index (HH) (88.32487%), RDW (87.95812%), and MCHD (86.66667%).

NPV for Hameed Index can't be calculated at all.

So, in relation to the old cut-off values available before Kerman Index 2 is the highest dependable index with 100% of Sensitivity and 65.09 % of Specificity which means that all β -TT cases will be correctly recognized and 34.90% of IDA cases will wrongly be diagnosed to have β-TT.

Kerman Index 1, RBCs count also have high discriminator function with high Sensitivity (100%, and 82.61%respectively) and specificity (61.02%, and 71.19% respectively).

The others had either low Specificity or Sensitivity which lead to a high number of false-positive or missed cases of β -T carriers respectively.

Discussion

In a 2014 study, **Vehapoglu et al. [13]** demonstrated that RBC count had only 70.5% specificity and 65.3% Youden's index. In the 290 children with microcytic anemia, 186 children (64.1%) had a high RBC count (RBC count > 5.0 10^{12} / L) at the time of diagnosis.

However, the frequency of high RBC count was 29.4% in children with IDA. It seems that RBC alone was not a reliable tool for distinguishing β-TT from IDA [**13**] and this is disagreeing with our result.

Similarly, in this study there are also indices with specificity more than 80% **Sirachainan et al. [14] (**100%), Huber-Herklotz Index (HH) (98.31%), RDW (94.35%), MDHL for male (84.35%), Ricerca et al index (82.49%), England and Fraser index (83.05%), and Keikhaei index (80.23%).

Their calculated normal values of MCHD and MDHL were very close to those published by **Telmissani et al. [15]**, although they found that mean and median values of MDHL in IDA were significantly lower than those in β-TT and normal values **[16].**

In **Adlekha et al. [17]** study, which done in 2013 on 88 cases with β-TT, they found that the highest Youden's index was obtained for RDWI (80%) followed by differentiating score (69%)

and further in decreasing order by MDHL, MI, and RBCs.

In their study, MDHL showed sensitivity of 77.2% and specificity of 85.1%, and DS showed sensitivity of 95% and specificity of 74%. This is in contrast to the observations made by **Telmissani et al. [15]**, in which MDHL showed sensitivity of 90% and specificity of 100%, whereas DS showed sensitivity of 83% and specificity of 81.4%. This can be attributed to the variations in the regional populations, resulting in a significant difference between the median MDHL of there and our study **[17].**

In our study, we noticed that Kerman Index 1 has Youden index (61.02%) which could be an most reliable discriminator index between β-TT and IDA.

This result is in concordance with result of **Cohan & Ramzi [18],** study in this study they found that Kerman index I with higher sensitivity 93%, specificity 87%, and Youden index 80% came out to be more valid in screening of beta-thalassemia minor and for Kerman index II the parameters were: sensitivity 76%, specificity 90%, and Youden index 66% in order **[18].**

In a 2009 study, which done in Iran on 154 patients with β-TT **Ehsani et al. [19]** showed that the superlative discrimination index according to Youden's value was (90.1%) for Mentzer index (90.1%), followed by (85.5%) for Ehsani et al. index. In **Ehsani et al. [19]** study indices were able to correctly diagnose 94.7% and 92.9% of cases, in that order, and both are simple to compute **[19]**.

Similar results (Mentzer index: sensitivity, 90.9%; specificity, 80.3%) were found by **Ghafouri et al. [20]**, while in our study MI show 100%

sensitivity but only 58.76% specificity and **Ehsani et al. [19]** index have 100% sensitivity and only 58.76%.

In **Batebi et al. [21]** study which done on 273 males with β-TT show that MI is the most reliable with Youden index 88%.

And this is similar to **Urrechaga [22]** study which done in Spain on 150 patients with β-TT show that MI is the most reliable index with Youden index 69%.

On the other hand, in the current study some indices, have less than 30% sensitivity MCHD has sensitivity equal to (26.09%), while **Sirachainan et al. [14],** Huber-Herklotz Index (HH), and RDW are not sensitive at all their sensitivity were (0.0%).

And specificity less than 30% in **Bordbar et al. [23]** (25.99%), and Shine and Lal (1.13%)), and Hameed Index has no specificity at all.

In current study as regard Youden index less than 30% we found it in **Bordbar et al. [23]** (25.99%), England and Fraser index (22.18%), Differentiating score for male, MCHD, Shine and Lal, and Hameed Index show the lowest Youden index (6.52%,4.06%,1.13%, and 1%, respectively). While Sirachainan et al (0.0%), Huber-Herklotz Index (HH) (-1.69%), and RDW (-5.65%). are not reliable at all and show the lowest Youden index.

Although RDW, a measure of the degree of variation in red cell size, has been informed to be a good discrimination index to differentiate between IDA and thalassemia minor [**24, 25]**, our results submit that this index may be confusing as all β-TT cases had an elevated level of RDW more than the cut-off value. When validity analysis was applied on RDW, this index had the lowest Youden's Index values.

This finding is closely similar to results of **Alfadhli et al. [12]** study.

Many studies had also informed that RDW alone is not sufficiently specific or sensitive enough to differentiate between microcytic anemias, as elevated values were not specific for IDA **[26, 27].**

In the current study, our results match with **Shen et al. [28]** study which done in china in which HH show low value of Youden index equal 15.8%.

In a 2006 study, which done in Kuwait on 153 confirmed cases of microcytic anemias; 47 patients with β-TT **AlFadhli et al. [12]** compared 9 indices in cases with microcytic anemia and calculated validity by using Youden's index. They showed that the highest Youden's index value was for England and Fraser index (98.2%) for correctly discriminating β-TT cases from IDA cases, the RBC count came second with Youden's Index of (78.8%), while RDWI rated at number 6 of the nine functions analyzed. whereas the Shine and Lal index was useless for distinguishing microcytic anemia with Youden index 50%. Finally, they concluded that the E&F index showed with great sensitivity and specificity to be the best discriminant function to differentiate between IDA and thalassemia minor cases **[12]** and this not match our results in that the E&F index doesn't show great sensitivity and specificity.

In a 2014 study, **Vehapoglu et al. [13]** demonstrated that the Mentzer index had the highest Youden's index for correctly recognizing β-TT cases and IDA cases at 81%. When the Mentzer index was computed, 91% of cases with microcytic anemia were correctly diagnosed. The Shine and Lal and England and Fraser indices had the lowermost Youden's index values of

10.2% and 51.4 %, respectively, and this match our results.

In a 2007 study, **Keikhaei et al. [29]** they found that Youden's index of RBC count and RDWI were the highest ones and they were the most reliable discrimination indices in differentiating β-TT from IDA in the older age group while for patients in the younger age group RBC and S & L were the most reliable discrimination indices on the other hand Mean Cell Hemoglobin Density (MCHD) and Mean Density of Hemoglobin per Liter (MDHL) did not show any diagnostic value, and this doesn't match our results in that S & L were the most reliable discrimination index in younger patients.

In this study, the RoC curve is generated to evaluate different indices and formulas. The formula will be more accurate and reliable when the area under the RoC curve is greater. The AUC of 1.0 represents the best differentiation while AUC of 0.5 represents the least valuable one **[6].**

In our study RBCs count as a discriminative function has the largest AUC equal 0.827 cm2, while RDW as a discriminative function show the smallest AUC equal 0.55cm2.

It is concerning that the cut-off values differed according to ethnicity, gender, and age. We found that most of the cut-off values for the published indices were not appropriate for the Egyptian population. In an endeavor to improve the trustworthiness of these indices, we predicted to establish our own values by using RoC curvatures.

We used RoC curves to evaluate each index and define more accurate cut-off values. Referring to the AUC value, we found that Sirdah et al index with Youden index 61.6% worked well in our children in differentiation between IDA and β-TT cases after adjusting

the new cutoff values form <27 to ≤28.97 with high sensitivity100% and specificity 61.58%.

Followed by Kerman Index 2, and Kerman Index 1 that has the same Youden index (61%), then (Ehsani et al index, and Srivastava index) their Youden index is (60.5%),

However, even when testing new cut-offs, RDW as a discriminative function remains the lowest index with Youden index (31.5%) after adjusting the new cut-off value from<13 to ≤17.5 with 60.87% sensitivity and 70.62% specificity.

This proposes RDW can't be a dependable discriminating index between β-TT and IDA cases in our population.

In **Sirachainan et al. [14]** study, which done in 2014 at Thailand on 111 school-age students with TT, their formula had the largest AUC with sensitivity and specificity of 84.6% and 87.5%, respectively. Therefore, a score >14 is suggestive of thalassemia trait, while a score ≤14 is suggestive of IDA **Sirachainan et al. [14]** which is relatively close to our new cut-off >11.395 is suggestive to β-TT.

At last, and to abridge the discoveries of our research, we may conclude that the differences between β - TT and IDA is based on:

- Mentzer index (MI) in β-TT ≤12.549, & in IDA>12.549

- Modified Mentzer index in β-TT ≤-0.451, & in IDA >-0.451
- RDWI in β-TT ≤177.22, & in IDA>177.22
- Shine and Lal in β-TT ≤766.13, & in IDA>766.13
- Srivastava and Bevington index in β-TT ≤4.233, & in IDA>4.233
- Green and King index in β-TT≤59.104, & in IDA>59.104

- Sirdah et al index in β-TT≤28.97, & in IDA>28.97
- Ehsani et al index in β-TT ≤13.7, & in IDA>13.7
- England and Fraser index in β-TT≤5.97, & in IDA>5.97
- Ricerca et al index in β-TT≤3.109, & in IDA>3.109
- MDHL in β-TT >1.518, & in IDA≤1.518
- MCHD in β-TT ≤0.316, & in IDA>0.319
- Keikhaei index in β-TT ≤17.465, & in IDA>17.465
- Sirachainan et al in β-TT >11.395, & in IDA≤11.395
- Bordbar et al in β-TT>126, & in IDA≤126
- Differentiating score for male in β-TT ≤-0.918, & in IDA >-0.918
- Differentiating score for female in β-TT≤-0.064, & in IDA>-0.064
- Huber-Herklotz Index (HH) in β-TT ≤22.992, & in IDA>22.991
- Kerman Index 1 in β-TT ≤252.7, & in IDA>252.7
- Kerman Index 2 in β-TT≤8.152, & in IDA>8.152
- Hisham Index (Hi) in β-TT ≤55.964, & in IDA >55.964
- Hameed Index in β-TT ≤3.57, & in IDA>3.57
- RDW in β-TT ≤17.5, & in IDA >17.5
- RBCs count in β-TT >5.1, & in IDA<5.1

Conclusion

The wide spectrum of β chain molecular alterations in the world and the degree of anemia may influence the discrimination function and reliability of RBC indices and formulas. The evaluation of iron status and measurement of HbA2 remain the most reliable investigations to

differentiate between β-TT and ID subjects.

Although one cannot reach a definitive diagnosis of IDA or βTT based merely on the discriminant functions, these simple calculations are potentially useful in screening patients with microcytic anemia. These indices are a useful tool in the doctor's guidance about the initial approach to be adopted, but do not relieve patient monitoring that may eventually require confirmatory tests to elucidate the strong suspicion initially raised by the application of these simple indices.

Furthermore, these formulas can be the only differential tool in situations where other specific confirmatory tests are not available.

None of the DFs shows the sensitivity and specificity of 100%.

The present study indicates that Kerman Index 1, Mentzer index (MI), Ehsani et al index, Modified Mentzer index, Kerman Index 2, and RBCs count are the highest reliable discriminator indices in differentiating of β-TT and IDA in sohag country.

Conversely Differentiating score for male, MCHD, Shine and Lal, Hameed Index, Sirachainan, et al, Huber-Herklotz Index (HH), and RDW as a discriminative function appear ineffective in our population.

The remainders suggest an moderate reliability in distinguishing β-TT and IDA in the population study.

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