

Evaluation of Twenty Four Discriminant Indices for Differentiating Beta-Thalassemia Trait from Iron Deficiency Anemia in Egyptians

Shaimaa Abdelmalik Pessar, MD

Faculty of Medicine, Ain Shams University - Cairo, Egypt

*Corresponding author: Shaimaa Abdelmalik Pessar, Clinical Pathology Department, Faculty of Medicine, Ain Shams University, Abbaseya, Cairo, Egypt, postal code: 11591. Email: shaimaamalik@med.asu.edu.eg. Orchid ID: orcid.org/0000-0002-8947-2324

Received: 10 February 2019

Accepted: 10 June 2019

Abstract

Background: Many Red Blood Cell (RBC) indices have been developed based on mathematical formulae to discriminate beta-thalassemia trait (β TT) from iron deficiency anemia (IDA). The latter two conditions represent the most common causes of microcytic hypochromic anemia in Egypt. This study aimed To evaluate the diagnostic reliability of 24 published discriminant indices for differentiating β TT from IDA in Egyptians.

Materials and Methods: A cross sectional study included a total of 166 subjects (108 IDA & 58 β TT) aged 1-18 years were recruited from Hematology laboratory of Pediatric Hospital, Ain Shams University, Cairo, Egypt. A full diagnostic algorithm was performed using complete blood count, hemoglobin electrophoresis by High Performance Liquid Chromatography (HPLC), iron profile and PCR detection of 22 mutations common for β TT. Twenty-four formulas were applied and their performance characteristics were calculated for each index.

Results: The highest accuracy (True positive + True negative/ All cases) & Youden's Index (Sensitivity+Specificity-100) were for Red Cell Distribution Width (RDWI) and Hameed index closely followed by Keikhaei index while the least performance was for RDW-SD, RDW-CV and Shine & Lal indices.

Conclusion The superiority of an index over another in distinguishing β TT from IDA allowed only partially better selection of cases warranting further confirmatory molecular studies. None of the studied formulae provided a surrogate test for Hb electrophoresis as mass screening.

Key words: Anemia, Beta-Thalassemia, Erythrocyte Indices, Iron deficiency.

Introduction

The two most frequent causes of microcytic anemia worldwide are beta thalassemia trait (β TT) and iron deficiency anemia (IDA) (1). In Egypt, both conditions represent a public health problem. About 64% of Egyptian children, especially from rural areas, have IDA (2). Also, the carrier rate of β TT ranges from 9-16.4% with an overall rate of 12.36% (3). Diagnosis of β TT often becomes perplexing due to its overlapping and similar features with IDA (4). Definitive methods for differential diagnosis between β TT and IDA include quantitative detection of HbA2 and DNA mutational analysis. While being accurate, these tests are too expensive and time-consuming for initial mass screening (5). Since screening will continue to be the cornerstone of the

strategies aimed at β -thalassemia control, attempts to develop effective and economic techniques for thalassemia screening have become essential in the countries with high prevalence of such diseases (6). Automated cell counters could provide a rapid, technically reliable method for effective population screening of β TT (7). Red cell indices have been used in a number of formulae, designed to discriminate cases of iron deficiency from cases of β TT, each has some degree of inaccuracy (8-25). A test is diagnostically valuable if it shows: superior sensitivity, specificity, efficiency, Youden's index (which is highly sensitive to the spectrum of the disease in a studied population), high positive predictive value, negative predictive value, positive likelihood ratio, and low negative likelihood ratio (26).

Measures of diagnostic accuracy are very sensitive to the characteristics of the population in which the test is evaluated. It is therefore of utmost importance to know how to interpret them as well as when and under what conditions to use them (27). This study intended to evaluate the diagnostic reliability of 24 published Differentiating Formulas (DFs) in detecting Egyptian children and adolescents who have β TT.

Materials and Methods

This cross sectional study was carried out on 280 microcytic hypochromic children and adolescent recruited from a total population of 1627 (17.2%) that presented to the outpatient clinics of Pediatric Hospital, Ain Shams University, Cairo, Egypt. Out of the 280 microcytic hypochromic children and adolescents, a total of 166 children and adolescents (108 IDA & 58 β TT) aged 1-18 years were enrolled in the study after procuring informed consents from their care givers. Out of the 166, 103 (62%) were males and 63 (38%) were females with a male to female ratio of 1.6:1. They were then divided into 108 IDA and 58 β TT according to the results of their iron profile, Hb A₂ quantitation by High Performance Liquid Chromatography (HPLC), and beta thalassemia gene mutation testing by Polymerase Chain Reaction (PCR).

All subjects underwent the following procedures:

- Complete blood count (Coulter Gen System 2, Beckman-Coulter, Miami, FL, USA).
- Hb A₂ quantitation (β TT short dual program, D10, Bio-Rad Laboratories, California, USA), D10 is a compact high performance liquid chromatograph designed for simple and fully automated measurement of HbA_{1c}, A₀, F and for the detection of abnormal Hbs.
- Serum iron and total iron binding capacity measure (Colorimetrically by Olympus

AU 600 autoanalyser, Olympus Diagnostics GmbH, Hamburg, Germany), and serum ferritin (radioimmunoassay by ARCHITECT, Abbott Diagnostics, Wiesbaden, Germany).

- Detection of β -globin gene mutations (Reverse hybridization PCR, β -Globin Strip—Assay MED, Vienna Lab Diagnostics, GmbH). The β -Globin StripAssay MED™ is based on the reverse-hybridization principle, and includes three successive steps: DNA is isolated from samples by a rapid and convenient procedure then β -globin gene sequences are in vitro amplified and biotin-labeled in a single (multiplex) amplification reaction. Finally, the amplification products are selectively hybridized to a test strip, which contains oligonucleotide probes (wild type- and mutant-specific) immobilized as parallel lines. Bound biotinylated sequences are detected using streptavidin-alkaline phosphatase and color substrates. The assay covers 22 mutations characteristic for Mediterranean area: -101[C→T], -87 [C→G], -30 [T→A], codon 5 [-CT], codon 6 [G→A] HbC, codon 6 [A→T] HbS, codon 6 [-A], codon 8 [-AA], codon 8/9 [+G], codon 15 [TGG→TGA], codon 27 [G→T] (knossos), IVS I-1 [G→A], IVS I-5 [G→C], IVS I-6 [T→C], IVS I-110 [G→A], IVS I-116 [T→G], IVS I-130 [G→C], codon 39 [C→T], codon 44 [-C], IVS II-1 [G→A], IVS II-745 [C→G], IVS II-848 [C→A]. The study was conducted in accordance with the stipulations of the local ethical and scientific committees of Ain Shams University; Egypt and the procedures respected the ethical standards in Helsinki declaration of 1964. The 24 formulas were calculated for all cases. (Table I) (8-25).

Statistical analysis

Quantitative variables were presented either as mean and standard deviation (SD) or median and range, and qualitative variables as number and percentage. Quantitative variables were compared using unpaired t-test; Analysis of data was

done on an IBM computer using the Statistical Package for the Social Sciences, version 12 (SPSS Inc., Chicago, IL, USA) and were evaluated at the 0.05 significance level. The diagnostic value of each formula was performed by calculating the Sensitivity, Specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV), Positive likelihood Ratio (LR+), Negative likelihood Ratio (LR-), Diagnostic Accuracy (efficiency); True positive + True negative/ All cases & Youden Index (Sensitivity+Specificity-100).

Results

Comparison of routine hematologic parameters are shown in Table II. Genetic mutations in β TT subjects: The four most common mutations were, in order of decreasing frequency, IVS-1-6 (T>C) (16/58, 27.6%), IVS-I-110 (G>A) (13/58,

22.4%), IVS-I-1 (G>A) (12/58, 20.7) and IVS-II-745 (C>G) (7/58, 12.1%) followed by other less frequent mutations such as IVS-II-848 (C>A) and codon 5 (-CT) (3/58, 5.2% for each), then IVS-II-1 (G>A) (2/58, 3.4%), and at last, -87 (C>G), and codon 39 (C4T) (1/58, 1.7% for each) (Figures 1 and 2). Diagnostic Performance of 24 calculated RBCs-indices-based formulae: The diagnostic performance of each formula is represented by its efficiency (diagnostic accuracy). Our study revealed that the highest accuracy (efficiency) and Youden index were for Red Cell Distribution Width (RDWI), Hameed index and Keikhaei index with the least performance and Youden index were for RDW-SD, Shine & Lal and Bessman & Feinstein indices (RDW-CV) (Table III and Figure 3).

Table (I): the discriminative indices with their respective cut-off values:

Index	Formula	β TT	IDA
Mentzer index (MI)	MCV/RBCs count	<13	>13
Modified Mentzer index		<12	>14
England and Fraser index (E&FI)	MCV-RBCs-(5xHb)-K K is calculated to be 8.4	<0	>0
Shine and Lal index (S&LI)	$MCV^2 \times MCH \times 0.01$	<1530	>1530
Green and King index (G&KI)	$MCV^2 \times RDW / 100 \times Hb$	<65	>65
Srivastava and Bevington index (S&BI)	MCH/RBCs	<3.8	>3.8
Red cell distribution width index (RDWI)	$MCV \times RDW / RBCs$	<220	>220
Ricerca et al index (RI)	RDW/RBCs	<3.3	>3.3
Red Blood Cell Count (RBCC)	RBCs count	>5	<5
Bessman and Feinstein index (B&FI)	RDW-CV	<14	>14
RDW-SD	RDW-SD	<46	>46
Keikhaei index (KI)	$Hb \times RDW \times 100 / (RBC)2 \times MCHC$	<21	>21
Ehsani et al index (EI)	$MCV - 10 \times RBC$	<15	>15
Sirdah et al index (SI)	$MCV - RBC - 3 \times Hb$	<27	>27
Mean Cell Hemoglobin Density (MCHD)	MCH/MCV	> 0.3045	< 0.3045
Telmissani et al index (MDHL)	MCHD \times RBC	>1.7M, >1.5F	<1.7M, <1.5F
Sirachainan et al	$1.5 Hb - 0.05 MCV$	>14	<14
Bordbar et al	$ 80 - MCV \times 27 - MCH $	>44.76	<44.76
Differentiating score (DS)	Male: $(0.096 \times MCV) + (0.415 \times RDW) - (0.139 \times RBCs) - 12.722$ Female: $(0.096 \times MCV) + (0.415 \times RDW) - 12.722$	<0.3095	>0.3095
Huber-Herklotz Index (HH)	$(MCH \times RDW \times 0.1 / RBC) + RDW$	<21	>23
Kerman index 1	$MCV \times MCH / RBCs$	<250	321-370
Kerman index 2	$MCV \times MCH / RBCs \times MCHC$	<8	10.5-13
Hisham index (Hi)	$(MCH \times RDW) / RBC$	< 67	≥ 67
Hameed index (Ha)	$(MCH \times Hct \times RDW) / (RBC \times Hb)^2$	< 220	≥ 220

Table (II): Comparison of hematological parameters between both disorders:

Variables	β TT (n=58)	IDA (n=108)	P
RBCs ($\times 10^6/\mu\text{L}$)	5.4 \pm 0.7	4.5 \pm 0.5	0.02 S
Hb (g/ dL)	10.6 \pm 1.5	9.4 \pm 1.8	0.03 S
HCT (%)	34 \pm 4.5	31 \pm 4	0.09 NS
MCV (fL)	63.9 \pm 4.5	65 \pm 6	<0.001 HS
MCH (pg)	19 \pm 1.8	19.7 \pm 3	0.06 NS
MCHC (g/dL)	31 \pm 1.7	30 \pm 2.7	0.1 NS
RDW (%)	17 \pm 3.4	19.5 \pm 4	<0.001 HS
HbA ₂ (%)	5.4 \pm 0.7	2.8 \pm 0.5	<0.001 HS
Serum iron ($\mu\text{g/dL}$)	39(28-60)	29(21-41)	0.07 NS
TIBC ($\mu\text{g/dL}$)	405 \pm 68	463 \pm 62	0.1 NS
Serum ferritin (ng/mL)	56(42-69)	12(7-20)	<0.001 HS

β TT: beta thalassemia trait, IDA: iron deficiency anemia, S: significant, NS: non significant, HS: highly significant, RBCs: red blood cells, Hb: hemoglobin, Hct: hematocrit, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration, RDW: red cell distribution width, TIBC: total iron binding capacity

Table (III): Performance criteria (sensitivity, specificity, PPV, NPV, LR+, LR-, accuracy and Youden index) for each discriminating index.

		Sensitivity	Specificity	PPV	NPV	+ve LR	-ve LR	Accuracy	YI
MI	β TT	68.96	63.88	50.63	88.46	1.9	0.48	65.66	32.84
	IDA	63.88	68.96	88.46	50.63	2.06	0.52		
Modified MI*	β TT	78.94	68.57	57.69	85.71	2.5	0.31	46.98	47.51
	IDA	68.57	78.94	85.71	57.69	3.25	0.4		
E&F Index	β TT	63.79	74.07	56.92	79.21	2.46	0.49	70.48	37.86
	IDA	74.07	63.79	79.21	56.92	2.04	0.41		
S&L Index	β TT	100	0	34.94	0	1	0	34.94	0
	IDA	0	100	0	34.94	0	1		
G&K Index	β TT	50	89.81	72.5	76.98	4.90	0.56	75.90	39.81
	IDA	89.81	50	76.98	72.5	1.8	0.2		
S&B Index	β TT	55.17	66.66	47.05	37.47	1.65	0.67	62.65	21.83
	IDA	66.66	55.17	37.47	47.05	1.49	0.6		
RDWI	β TT	70.69	83.33	69.49	84.11	4.24	0.35	78.91	54.02
	IDA	83.33	70.69	84.11	69.49	2.84	0.23		
RI	β TT	65.51	82.41	66.66	81.65	3.72	0.42	76.50	47.92
	IDA	82.41	65.51	81.65	66.66	1.0	0.27		
RBC count	β TT	67.24	71.29	55.71	80.21	2.34	0.46	69.88	38.53
	IDA	71.29	67.24	80.21	55.71	2.17	0.43		
B&F Index	β TT	5.17	96.29	42.86	65.41	1.39	0.98	64.46	1.46
	IDA	96.29	5.17	65.41	42.86	1.02	0.72		
RDW-SD	β TT	10.34	87.04	30	46.38	0.8	1.03	60.24	-2.62
	IDA	87.04	10.34	46.38	30	0.97	1.25		
KI	β TT	58.62	89.81	75.55	80.16	5.75	0.46	78.91	48.43
	IDA	89.81	58.62	80.16	75.55	2.17	0.17		
EI	β TT	65.52	63.89	49.35	77.53	1.81	0.54	64.46	29.41
	IDA	63.89	65.52	77.53	49.35	1.85	0.55		
SI	β TT	46.55	84.26	61.36	74.59	2.96	0.63	71.08	30.81
	IDA	84.26	46.55	74.59	61.36	1.58	0.34		
MCHD	β TT	74.14	49.07	43.88	77.94	1.46	0.53	57.83	23.21
	IDA	49.07	74.14	77.94	43.88	1.90	0.69		
MDHL	β TT	62.07	83.33	66.67	80.36	3.72	0.46	75.90	45.4
	IDA	83.33	62.07	80.36	66.67	2.20	0.27		
Sirachainan et al	β TT	24.14	89.81	56	68.79	2.37	0.84	66.87	13.95
	IDA	89.81	24.14	68.79	56	1.18	0.42		
Bordbar et al	β TT	94.83	24.07	40.14	89.66	1.25	0.21	48.79	18.9

	IDA	24.07	94.83	89.66	40.14	4.65	0.8		
DS	βTT	68.96	72.22	57.14	81.25	2.48	0.43	71.08	41.18
	IDA	72.22	68.96	81.25	57.14	2.33	0.4		
HH Index**	βTT	32.43	95.79	75	78.45	7.7	0.7	62.05	28.22
	IDA	95.79	32.43	78.45	75	1.42	0.13		
Kerman index 1***	βTT	68	36.67	47.22	57.89	1.07	0.87	33.73	4.67
	IDA	36.67	68	57.89	47.22	1.15	0.93		
Kerman index 2****	βTT	80.49	42.62	48.53	76.47	1.40	0.46	35.54	23.11
	IDA	42.62	80.49	76.47	48.53	2.18	0.71		
Hisham index (Hi)	βTT	67.24	81.48	66.10	82.24	3.63	0.40	76.50	48.72
	IDA	81.48	67.24	82.24	66.10	2.49	0.27		
Hameed index (Ha)	βTT	70.69	83.33	69.49	84.11	4.24	0.35	78.91	54.02
	IDA	83.33	70.69	84.11	69.49	2.84	0.24		

*MMI had 58 indeterminate cases (12-14).** H-H index had 34 indeterminate cases (21-23).

Kerman 1 had 22 indeterminate cases (250-320).*Kerman 2 had 26 indeterminate cases (8-10.4).

MI: Mentzer index, E&F: England and Fraser index, S&L: Shine and Lal index, G&K: Green and King index, S&B: Srivastava and Bevington index, RDWI: Red Cell Distribution Width index, RI: Ricerca index, B&F: Bessman and Feinstein index, RDW-SD: Red Cell Distribution Width-standard deviation, KI: Keikhaei index, EI: Ehsani index, SI: Sirdah index, MCHD: Mean Cell Hemoglobin Density, MDHL: Mean Density Of Hemoglobin Per Liter Of Blood or Telmissani et al index, DS: Differentiating score, HH: Huber-Herklotz index, βTT: beta thalassemia trait, IDA: iron deficiency anemia, PPV: Positive Predictive value, NPV: Negative Predictive value, LR+: Positive Likelihood ratio, LR-: Negative Likelihood ratio, YI: Youden index.

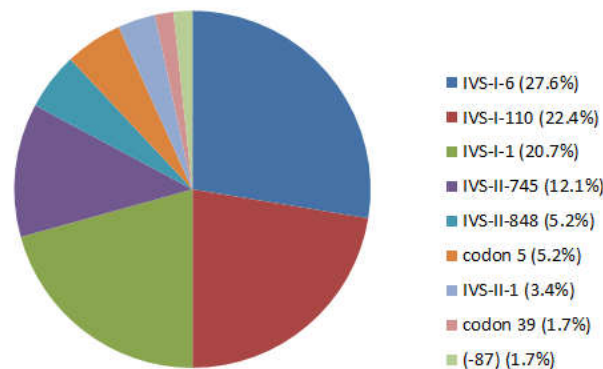


Figure 1: β thalassemia mutations frequency in carriers

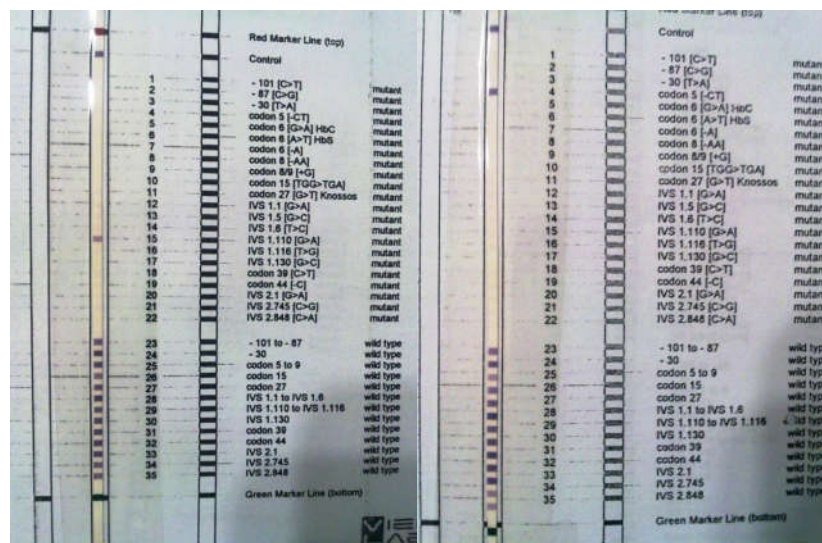


Figure 2: Lt: heterozygosity for IVS 1.110, Rt: heterozygosity for codon 5

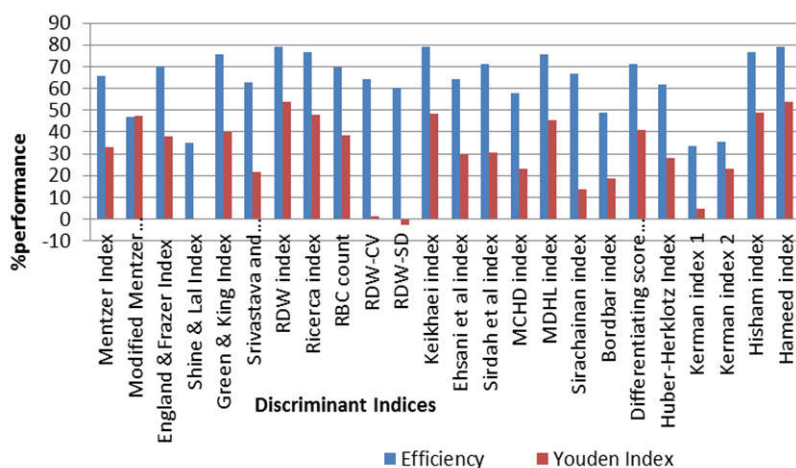


Figure 3: Diagnostic Performance of The Studied Discriminant Indices

Discussion

The high carrier rate of β -thalassemia in Egypt is confounded by the phenotypic and genotypic heterogeneity attributed to its position as a liaison between the middle east and the Mediterranean basin. Given the high prevalence of each of IDA and β TT in the population; simple, cost-effective and reliable screening tools for differentiation between both disorders are needed to avoid the use of the more laborious and expensive genetic studies. Many studies have evaluated the efficiency and discriminative power of various RBC indices and discriminating formulas which have come into interest. Individual hematological parameters as; Hb, RBCs count, Hb A2 and ferritin were significantly higher in β TT than IDA. Meanwhile, MCV and RDW were significantly lower in β TT than IDA. Reduction of MCV and MCH values in β TT did not correlate with the degree of anemia, similar findings were reported in Nesa et al (28) and Rathod et al (29). In this study, clinical, laboratory and genetic diagnoses were established for all the 166 cases; 108 IDA and 58 β TT. Then, the twenty four indices were retrospectively

calculated for all cases and each index was evaluated for its discriminating power between IDA and β TT according to sensitivity, specificity, PPV, NPV, LR+ve, LR-ve, accuracy and Youden index. The reliability of various formulae studied in this work gave performance characteristics unique to their respective populations. In Kurdistan/Iraq, Hameed index and Hisham index achieved the highest youden indices followed by RDWI and Keikhaei index with the least reliable being S&L index. (25). High reliability of RDWI was reported by Nesa et al. (28) in Bangladesh and Sirdah et al. (18) in the Palestinian population together with their formula and G&K index. Alfadhli et al. (30) in Kuwait concluded that the E&F index was the best discriminant while the S&L index was found ineffective in the differentiation between IDA and β TT cases. Urrechaga (31) in Spain and Ntaios et al. (32) in Greece found that G&K index ranked the best with a Youden index of 80.9% and 70.86% respectively. In Pakistan, Niazi et al. (7) concluded that the accuracy and Youden index were highest for RDWI followed by Mentzer and S&L index while, Ehsani and colleagues (17) showed

Mentzer as the best discrimination index followed by their new index in Pakistani patients. In India, Dhara and Harsh (33) introduced the RDW-SD as the best for distinguishing between IDA and β TT. They also considered both the RBC count and E&F index to be useful functions for that purpose while the RDW-CV to be ineffective whereas, Adlekha, et al. (4) found that RDWI and DS were of higher sensitivity and specificity than MDHL and MI in predicting β TT. In Turkey, similar results regarding the high reliability of RDWI were reported by Demir et al. (34) together with RBC count. Also, Beyan et al. (35) who concluded that none of the discriminant formulations was superior to RBC Count. While, Vehapoglu et al. (1) found that the highest efficiency was for the Mentzer index (91%) followed by the Ehsani et al. index (84.8%) then RBC count (83.4%) in children. Contrarily, Aslan and Altay (36) concluded that high RBC count cannot be regarded as a reliable preliminary parameter in differentiating IDA from thalassemia in young children. Okan et al. (37) reported that S&L and G&K indices offered the best discrimination. In Iran, the same contradictory results were reported; Rahim & Keikhaei (38) proved that RBC count and RDWI were the most reliable discrimination indices in patients older than 10 years while S&L index had the highest youden index in those younger than 10 years. Also, Keikhaei (19) concluded that his index, G&K index, RDWI and E&F index are the highest reliable. Similarly, Moghaddam et al. (5) showed that the highest reliability was for Green & King, along with Ricerca, and England & Fraser indices. Batebi et al. (49) revealed that the E&F index had superior sensitivity over S&L index, Mentzer index and traditional red cell indices. Contrarily, Ghafouri et al. (40) recommended Mentzer index. Also, Rajabiani et al. (41) concluded that Mentzer and MDHL achieved the best performance while E&F and S&L was of

low sensitivity. In Brazil, high reliability of RDWI was reported by Matos et al. (42) together with G&K index. Also, Lima et al. (43) concluded that G&K index is better than RDW in differentiating IDA from β TT. In contrast to Melo et al. (44) who proposed that β TT was better distinguished from IDA by RBC count and E&F index. Shen et al. (45) studied Chinese children and showed that the highest reliability was for Green & King, along with Ricerca, and England & Fraser indices, while Srivastava and Shine & Lal indices showed the lowest reliability. Ferrara et al. (46) studied Italian children and found that the highest sensitivity was for RDWI, while the highest specificity and Youden index were for E&F index and the highest efficiency was for G&K index. In Egypt, the present study revealed that the highest reliability was for RDWI and Hameed index closely followed by Keikhaei index with the same efficiency. Also, both Hisham and Ricerca indices achieved the next highest accuracy. The differentiating indices of Telmissani, Green & king, Sirdah, differentiating score, England & Fraser and RBCs count gave moderate reliability while Sirachainan et al., Mentzer, Ehsani, Srivastava & Bevington, Huber-Herklotz, MCHD, Bordbar et al, Kerman 1 and Kerman 2 gave low reliability with the least performance was for RDW-SD, Shine & Lal, and Bessman & Feinstein indices (RDW-CV). According to this study, RBCs count alone was not reliable in discriminating between IDA and β TT as IDA in children might be associated with erythrocytosis. It also increased at the initiation of iron therapy and decreased by the end of therapy. A similar observation was made by Rathod et al. (29) who concluded that RBC & RDW and their related formulas (G&K and Ricerca indices) have bad discriminative function compared with cell counter-based parameters particularly MCV and MCH and their related formulas (S&L, Srivastava index, and Mentzer indices) for

the diagnosis of β TT. The role of RDW as an auxiliary parameter in the differentiation of various anemias is controversial. In some studies (47, 48), a significant difference was observed for RDW between patients with IDA and β TT, while in others this parameter presented low diagnostic accuracy (30, 34, 42, 43, 46, 49-52). However, some studies did not exclude presence of interfering factors or concomitant diseases that could influence RDW performance which is considered a drawback. The four most common mutations found among β TT population; IVS-I-6 (T>C), IVS-I-110 (G>A), IVS-I-1 (G>A) and IVS-II-745 (C>G) followed by other less frequent mutations such as IVS-II-848 (C>A) and codon 5 (-CT), then IVS-II-1 (G>A), -87 (C>G) and codon 39 (C>T) were in concordance with a previous study by Waye et al. (53) who combined data from 5 published surveys of Egyptian β -thalassemia mutations. From the previous data, IVS 1.110 was found to be the most frequent mutation in studies conducted predominantly on β -thalassemia major (54-59). Meanwhile, IVS 1.6 was found to be the most frequent mutation in studies conducted predominantly on β thalassemia carriers (with or without β thalassemia intermedia) like this study (60-62). Of all the above literature, this study was the only work to collectively apply twenty four differentiating indices, which when assessed in Egyptian population, the newer discriminant indices e.g. RDWI achieved better performance than the older indices (England and Fraser, Mentzer, Green and King) which may be partly explained by the younger age group as proposed by Hoffmann et al. (63) who demonstrated high variation in the performance of discriminant indices according to age and geographical region, but not the type of hematology analyzer, which considered important factors determining the diagnostic utility of these indices. Also, Hoffmann et al. (63) have objectively shown the superiority of the M/H ratio

over other indices. Despite its high performance, the M/H ratio cannot be used for making a final diagnosis of thalassemia trait. Hussain et al. (64) and Urrechaga et al. (65) proposed formulae that require the availability of a specific generation of hematology analyzers which can give the percentage of hypochromic red cells and the percentage of microcytic red cells and claimed that these formulae were able to efficiently screen patients and confirm β TT. The controversies in relevant literature with different performances for the same index between different regions is attributed to different thalassemia mutations affecting various hematological parameters, degree of severity of IDA, population selection criteria and possibility of concomitant clinical condition. Therefore, larger population based genetic studies correlating between formulae and each of mutations detected in the studied region together with studies proposing new formulae or adopting new cut-offs for existing formulas may prove to be more useful. In conclusion, none of these CBC based indices was completely satisfactory per se in differentiating between β TT or IDA. Studies have shown that these formulas properly identify only 61–91% of the patients with microcytic anemia due to β TT or IDA.

Compliance with ethical standards

Informed consent was taken from participants or from their legal guardians before enrollment into the study.

All procedures performed in this study involving participants were in accordance with the ethical standards approved by the local ethical committee of the Ain Shams University Faculty of Medicine.

Funding

The author did not receive any research grant, this work was funded by the author herself.

Acknowledgement

I would like to express my deep gratitude to Dr. Soha Raouf Youssef, Professor of clinical pathology, Ain Shams University, Cairo, Egypt, for her assistance, great

support and comments that greatly improved the manuscript..

Conflict of interest

Author declared no conflict of interest.

References

1. Vehapoglu A, Ozgurhan G, Demir AD, Uzuner S, Nursoy MA, Turkmen S, et al. Hematological indices for differential diagnosis of beta thalassemia trait and iron deficiency anemia. *Anemia* 2014; 2014:576738-576742.
2. Al Ghwass MM, Halawa EF, Sabry SM, Ahmed D. Iron deficiency anemia in an Egyptian pediatric population: A cross-sectional study. *Ann Afr Med* 2015;14 (1):25-31.
3. El-Tohamy MK, Eissa AN, El Dorry G, Nasser M, Donia A, Ramzy S. Prevalence of thalassemia carriers among Egyptian school students. *Sci Med J ESCME* 2003; 15: 4-9.
4. Adlekha S, Chadha T, Jaiswal RM, Singla A. Screening of β -thalassaemia trait by means of red cell indices and derived formulae. *Med J Dr D Y Patil Univ* 2013; 6 (1):71-74
5. Miri-Moghaddam E, Sargolzaie N. Cut off determination of discrimination indices in differential diagnosis between iron deficiency anemia and β -thalassemia minor. *Int J Hematol Oncol Stem Cell Res* 2014; 8:1–6.
6. Sachdev R, Dam A, Tyagi G. Detection of Hb variants and hemoglobinopathies in Indian population using HPLC: Report of 2600 cases. *Indian J pathol microbial* 2010; 53 (1): 57-62.
7. Niazi M, Tahir M, Raziq F, Hameed A. Usefulness of red cell indices in differentiating microcytic hypochromic anemias. *Gomal J Med Sci* 2010;8:125-129.
8. England JM, Fraser PM. Differentiation of iron deficiency from thalassemia trait by routine blood-count. *Lancet* 1973;1(7801):447-449.
9. Mentzer WC. Differentiation of iron deficiency from thalassemia trait. *Lancet* 1973; 1 (7808):882-889.
10. Srivastava PC, Bevington JM. Iron deficiency and/or thalassemia trait. *Lancet* 1973; 1 (7807):832-835.
11. Shine I, Lal S. A strategy to detect beta thalassemia minor. *Lancet* 1977; 1(8013):692–694.
12. Green R, King R. A new red blood cell discriminant incorporating volume dispersion for differentiating iron deficiency anemia from thalassemia minor. *Blood Cells* 1989; 3:481–495.
13. Ricerca BM, Storti S, Onofrio G, Mancini S, Vittori M, Campisi S, et al. Differentiation of iron deficiency from thalassemia trait: A new approach. *Haematologica* 1987; 72(5):409–413.
14. Bessman JD, Feinstein DI. Quantitative anisocytosis as a discriminant between iron deficiencies and thalassemia minor. *Blood* 1979;53(2):288–293.
15. Jayabose S, Giamelli J, Levondoglu Tugal O, Sandoval C, Ozkaynak F, Visintainer P. Differentiating iron deficiency anemia from thalassemia minor by using an RDW-based index. *J Pediatr Hematol Onclo* 1999; 21(4):314-319.
16. Telmissani OA, Khalil S, Roberts GT. Mean density of hemoglobin per liter of blood: a new hematologic parameter with an inherent discriminant function. *Lab Hematol* 1999; 5:149–152.
17. Ehsani MA; Shahgholi E; Rahiminejad MS; Seighali F; Rashidi A. A new index for discrimination between iron deficiency anemia and beta-thalassemia minor: results in 284 patients. *Pak J Biological Sci* 2009; 12(5):473-475.
18. Sirdah M, Tarazi I, Al Najjar E, Al Haddad R. Evaluation of the diagnostic reliability of different RBC indices and formulas in the differentiation of the beta-thalassemia minor from iron deficiency in Palestinian population. *Int J Lab Hematol* 2008; 30 (4): 324–330.
19. Keikhaei B. A new valid formula in differentiating iron deficiency anemia from β -thalassemia trait. *Pakist J Med Sci* 2010; 26: 368–373.

20. Sirachainan N, Iamsirirak P, Charoenkwan P, Kadegasem P, Wongwerawattanakoon P, Sasanakul W, Chansatitporn N and Chuansumrit A. New Mathematical Formula For Differentiating Thalassemia Trait And Iron Deficiency Anemia In Thalassemia Prevalent Area: A Study In Healthy School-Age Children. *Southeast Asian J Trop Med Public Health* 2014; 45:1-9.
21. Bordbar E, Taghipour M, Zucconi B. Reliability of Different RBC Indices and Formulas in Discriminating between β -Thalassemia Minor and other Microcytic Hypochromic Cases. *Mediterr J Hematol Infect Dis* 2015; 7(1): e2015022-e2015027.
22. Fairbanks VF, Hines JD, Mazza JJ, Hocking WG. Chapter 2 The Anemias. In: Joseph J Mazza, manual of clinical hematology. Little, brown & co, Boston, New York, Toronto, London 1995;page 17.
23. Huber AR, Ottiger C, Risch L, Regenass S, Hergersberg M, Herklotz R. Thalassämie-syndrome: klinik und diagnose [Syndromes thalassémiques: clinique et diagnostic]. *Schweiz Mediz Forum* 2004; 4:947–952.
24. Kohan N., Ramzi M. Evaluation of Sensitivity And Specificity Of Kerman Index I And II In Screening Beta Thalassemia Minor. *Sci J Iran Blood Transfus Org (Khoon)* 2008; 4(4) : 297 - 302.
25. Hisham A.Getta, Hadeel A. Yasseen, Hameed M. Said. Hi & Ha, are new indices in differentiation between Iron deficiency anemia and beta-Thalassaemia trait /A Study in Sulaimani City-Kurdistan/Iraq. *IOSR-JDMS* 2015; 14(7):67-72
26. Youden WJ. Index for rating diagnostic tests. *Cancer* 1950; 3(1):32–35.
27. Šimundić A. Measures of Diagnostic Accuracy: Basic Definitions. *EJIFCC* 2009; 19(4): 203–211.
28. Nesa A, Abu Tayab , Sultana T, Khondker L, Rahman Q, Anwarulkarim, et al. RDWI is better discriminant than RDW in differentiation of iron deficiency anaemia and β thal trait. *Bangladesh J Child Health* 2009; 33(3): 100-103.
29. Rathod DA, Kaur A, Patel V, Patel K, Kabrawala R, Patel Vet al. Usefulness of cell counter–based parameters and formulas in detection of beta–thalassemia trait in areas of high prevalence. *Am J Clin Pathol* 2007;128(4):585–589.
30. Alfadhli SM, Al Awadhi AM, Alkhaldi AD. Validity assessment of nine discriminant functions used for the differentiation between iron deficiency anemia and thalassemia minor. *J Trop Pediatr* 2007; 53(2):93–97.
31. Urrechaga E. Dicriminant value of % microcytic/%hypochromic ratio in the differential diagnosis of microcytic anemia. *Clin Chem Lab Med* 2008;46(12):1752–1728.
32. Ntaios G, Chatzinikolaou A, Saouli Z, Girtovitis F, Tsapanidou M, Kaiafa G, et al. Discrimination indices as screening tests for β –thalassemic trait. *Ann Hematol* 2007;86(7):487–491.
33. Dhara P, Harsh A. Discriminant Functions In Distinguishing Beta Thalassemia Trait and Iron Deficiency Anemia: The value of the RDW-SD. *Internet J Hematol* 2011; 7 (2): 19-24.
34. Demir A, Yarali N, Fisgin T, Duru F, Kara A: Most reliable indices in differentiation between thalassemia trait and iron deficiency anemia. *Pediatr Int* 2002; 44(6):612–616.
35. Beyan C, Kaptan K, Ifran A. Predictive value of discrimination indices in differential diagnosis of iron deficiency anemia and beta- thalassemia trait. *Eur J Haematol* 2007;78(6):524–526.
36. Aslan D, Altay C. Incidence of high erythrocyte count in infants and young children with iron deficiency anemia: re-evaluation of an old parameter. *J Pediatric Hematol/Oncol* 2003;25(4): 303–306.
37. Okan V, Cigiloglu A, Cifci S, Yilmaz M, Pehlivan M. Red cell indices and functions differentiating patients with the β -thalassaemia trait from those with

- iron deficiency anaemia. *J Int Med Res* 2009; 37:25–30.
38. Rahim F, Keikhaei B, Better differential diagnosis of iron deficiency anemia from beta-thalassemia trait. *Turk J Hematol* 2009; 26(3):138–145.
 39. Batebi A, Pourreza A, Esmailian R. Discrimination of beta-thalassemia minor and iron deficiency anemia by screening test for red blood cell indices. *Turk J Med Sci* 2012; 42 (2): 275-280.
 40. Ghafouri M, Sefat L, and Sharifi L. Comparison of cell counter indices in differentiation of beta thalassemia trait and iron deficiency anemia. *Scientific J Iranian Blood Transfus Organ* 2006; 2(7): 385–389.
 41. Rajabiani A, Heshmati P, Ghafouri M. Mean Density of Hemoglobin, a better discriminator of iron deficiency anemia from thalassemia minor. *MJIRC* 2005; 8: 2-9.
 42. Matos JF, Dusse LM, Stubbett RV, Ferreira MR, Coura-Vital W, Fernandes APS et al. Comparison of discriminative indices for iron deficiency anemia and beta thalassemia trait in a Brazilian population. *Hematology* 2013; 18(3):169-174.
 43. Lima CSP, Reis ARC, Grotto HZW, Saad STO, Costa FF. Comparison of red cell distribution width and a red cell discriminant function incorporating volume dispersion for distinguishing iron deficiency from beta thalassemia trait in patients with microcytosis. *São Paulo Med J* 1996; 114(5): 1265-1269.
 44. Melo MR, Purini MC, Cancado RD, Kooro F, Chiattoni CS. The use of erythrocyte (RBC) indices in the differential diagnosis of microcytic anemias: is it an approach to be adopted? *Rev Ass Med Brasil* 2002; 48(3):222–224.
 45. Shen C, Jiang YM, Shi H, Liu J, Zhou W, Dai Q, et al. Evaluation of indices in differentiation between iron deficiency anemia and beta-thalassemia trait for Chinese children. *J Pediatr Hematol Oncol* 2010; 32(6):e218-222.
 46. Ferrara M, Capozzi L, Russo R, Bertocco F, Ferrara D. Reliability of red blood cell indices and formulas to discriminate between beta thalassemia trait and iron deficiency in children. *Hematology* 2010; 15(2): 112-115.
 47. Aslan D, Gumruk F, Gurgey A, Altay C. Importance of RDW value in differential diagnosis of hypochrome anemias. *Am J Hematol* 2002; 69(1): 31-33.
 48. Yermiahu T, Ben-Shalom M, Porath A, Vardi H, Boantza A, Mazor D, et al. Quantitative determinations of microcytic-hypochromic red blood cell population and glycerol permeability in iron deficiency anemia and beta thalassemia minor. *Ann Hematol* 1999; 78(10): 468-471.
 49. Eldibany MM, Totonchi KF, Joseph NJ, Rhone D. Usefulness of certain red blood cell indices in diagnosing and differentiating thalassemia trait from iron deficiency anemia. *Am J Clin Pathol* 1999; 111(5): 676-682.
 50. Janel A, Roszyk L, Rapatel C, Mareynat G, Berger MG, Serre-Sapin A. Proposal of a score combining red blood cell indices for early differentiation of beta-thalassemia minor from iron deficiency anemia. *Hematology* 2011; 16(2): 123-127.
 51. Lafferty JD, Crowther MA, Ali MA. The evaluation of various mathematical RBC indices and their efficacy in discriminating between thalassemic and non-thalassemic microcytosis. *Am J Clin Pathol* 1996; 106(2): 201-215.
 52. Tillyer ML, Tillyer CR. Use of red cell distribution width and erythrocyte zinc protoporphyrin in differential diagnosis of α and β thalassaemia and iron deficiency. *J Clin Pathol* 1994; 47(3): 205-208.
 53. Waye J, Bory S, Eng B, Patterson M, Chui D, Badr El-Din O, et al. Spectrum of β -thalassemia mutations in Egypt. *Hemoglobin* 1999; 23 (3): 255-261.
 54. Hussein G, Fawzy M, El-Serafi T, Ismail E, El-Metwally D, Saber M, et al. Rapid detection of β -thalassemia alleles in Egypt using naturally or amplified created

restriction sites and direct sequencing: A step in disease control. *Hemoglobin* 2007; 31 (1): 49-62.

55. El-Beshlawy A, Ragab L, Hussein I, El-Tagui M. Genotypes, phenotypes in β -thalassemia and sickle cell anemia in Egypt. *JAC* 1995; 6(2): 147-151.

56. Omar A, Abdel Karim E, Gendy WE, Marzouk I, Wagdy M. Molecular basis of beta-thalassemia in Alexandria. *Egypt J Immunol*. 2005; 12 (1): 15-24.

57. Gaafar T, El-Beshlawy A, Aziz M, Abdelrazik H. Rapid screening of β -globin gene mutations by real-time PCR in Egyptian thalassemia children. *Afr J Health Sci* 2006; 13: 3-4.

58. Naja R, Kaspar H, Shbalko H, Chakar N, Makhoul N, Zalloua P. Accurate and rapid prenatal diagnosis of the most frequent East Mediterranean β -thalassemia mutations. *Am J Hematol* 2004; (75): 220-224.

59. Tadmouri G. Deniz: The electronic database for β -thalassemia mutations in the Arab World. *Saudi Med J* 2003; 24(24): 1192-1198.

60. Shams El-Din A, Gaafar M, El-Beshlawy A, Sheba H, Ali H. Molecular characterization of non-transfusion dependant β -thalassemia. *Egypt J Lab Med* 1998; 10 (1): 165-185.

61. Hussein I, El-Beshlawy A, Aboul Ezz E, El-Hadidi S. Screening for β -thalassemia mutations utilizing buccal cells. *Egypt J Ped* 2000; 17 (2): 139-151.

62. El-Gawhary S, El-Shafie S, Niazi M, Aziz M, El-Beshlawy A. Study of β -thalassemia mutations using the polymerase chain reaction-amplification refractory mutation system and direct sequencing techniques in a group of Egyptian thalassemia patients. *Hemoglobin* 2007; 31 (1): 63-69.

63. Hoffmann JJ, Urrechaga E, Aguirre U. Meta-analysis of discriminant indices for microcytic anemia. *Clin Chem Lab Med* 2015; 53(12): 1883-1894.

64. Hussain Z, Malik N, and Chughtai A. Diagnostic Significance of red cell

indices in beta thalassemia trait. *Biomedica* 2005; 21:129-131.

65. Urrechaga E, Borque L, Escanero JF. The role of automated measurement of red cell subpopulations on the Sysmex XE 5000 analyzer in the differential diagnosis of microcytic anemia. *Int J Lab Hematol* 2011; 33: 30-6.