Original Article *Iran J Ped Hematol Oncol. 2019, Vol 9.No 3, 135-146*

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

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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 (βTT) 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 βTT from IDA in Egyptians.
Materials and Methods: A cross sectional study included a total of 166 subjects (108 IDA & 58 βTT) 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 βTT. 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 βTT 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 (βTT) 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 βTT ranges from 9-16.4% with an overall rate of 12.36% (3). Diagnosis of βTT often becomes perplexing due to its overlapping and similar features with IDA (4). Definitive methods for differential diagnosis between βTT 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 βTT (7). Red cell indices have been used in a number of formulae, designed to discriminate cases of iron deficiency from cases of βTT, 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).

[DOI: 10.18502/ijpho.v9i3.1163]

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 βTT.

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 βTT) 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 βTT according to the results of their iron profile, Hb A_2 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 A_2 quantitation (βTT 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 HbA1c, Ao, 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], codo 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

[DOI: 10.18502/ijpho.v9i3.1163]

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 βTT 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	βTT	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^2xMCHx0.01	<1530	>1530
Green and King index (G&KI)	MCV^2xRDW/100xHb	<65	>65
Srivastava and Bevington index (S&BI)	MCH/RBCs	<3.8	>3.8
Red cell distribution width index (RDWI)	MCVxRDW/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×RDW×100/ (RBC)2×MCHC	<21	>21
Ehsani et al index (EI)	MCV–10×RBC	<15	>15
Sirdah et al index (SI)	MCV–RBC–3×Hb	<27	>27
Mean Cell Hemoglobin Density (MCHD)	MCH/MCV	> 0.3045	< 0.3045
Telmissani et al index (MDHL)	MCHD×RBC	>1.7M, >1.5F	<1.7M, <1.5F
Sirachainan et al	1.5 Hb-0.05 MCV	>14	<14
Bordbar et al	$\lvert 80\text{-}MCV \rvert \times \lvert 27\text{-}MCH \rvert$	>44.76	<44.76
Differentiating score (DS)	Male: (0.096xMCV)+(0.415xRDW)-(0.139xRBCs)-12.722 Female: (0.096xMCV)+(0.415xRDW)-12.722	<0.3095	>0.3095
Huber-Herklotz Index (HH)	(MCH × RDW × 0.1/RBC)+ RDW	<21	>23
Kerman index 1	MCVxMCH/RBCs	<250	321-370
Kerman index 2	MCVxMCH/RBCsxMCHC	<8	10.5-13
Hisham index (Hi)	(MCH×RDW)/RBC	< 67	≥67
Hameed index (Ha)	$(MCH \times Hct \times RDW)/ (RBC \times Hb)^2$	< 220	≥220

[DOI: 10.18502/ijpho.v9i3.1163]

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

Variables	βTT (n=58)	IDA (n=108)	P
RBCs (x10^6/uL)	5.4±0.7	4.5±0.5	0.02 S
Hb (g/ dL)	10.6±1.5	9.4±1.8	0.03 S
HCT (%)	34±4.5	31±4	0.09 NS
MCV (fL)	63.9±4.5	65±6	<0.001 HS
MCH (pg)	19±1.8	19.7±3	0.06 NS
MCHC (g/dL)	31±1.7	30±2.7	0.1 NS
RDW (%)	17±3.4	19.5±4	<0.001 HS
HbA_2 (%)	5.4±0.7	2.8±0.5	<0.001 HS
Serum iron (µg/dL)	39(28-60)	29(21-41)	0.07 NS
TIBC (µg/dL)	405±68	463±62	0.1 NS
Serum ferritin (ng/mL)	56(42-69)	12(7-20)	<0.001 HS

βTT: 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	βTT	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*	βTT	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	βTT	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	βTT	100	0	34.94	0	1	0	34.94	0
	IDA	0	100	0	34.94	0	1		
G&K Index	βTT	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	βTT	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	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	0.23		
RI	βTT	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	βTT	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	βTT	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	βTT	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	βTT	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	βTT	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	βTT	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	βTT	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	βTT	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	βTT	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	βTT	94.83	24.07	40.14	89.66	1.25	0.21	48.79	18.9

[DOI: 10.18502/ijpho.v9i3.1163]

	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.

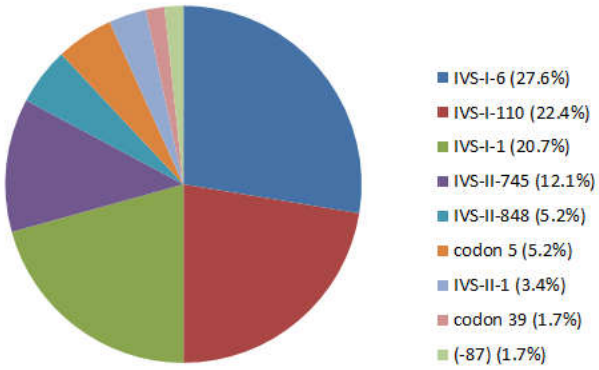
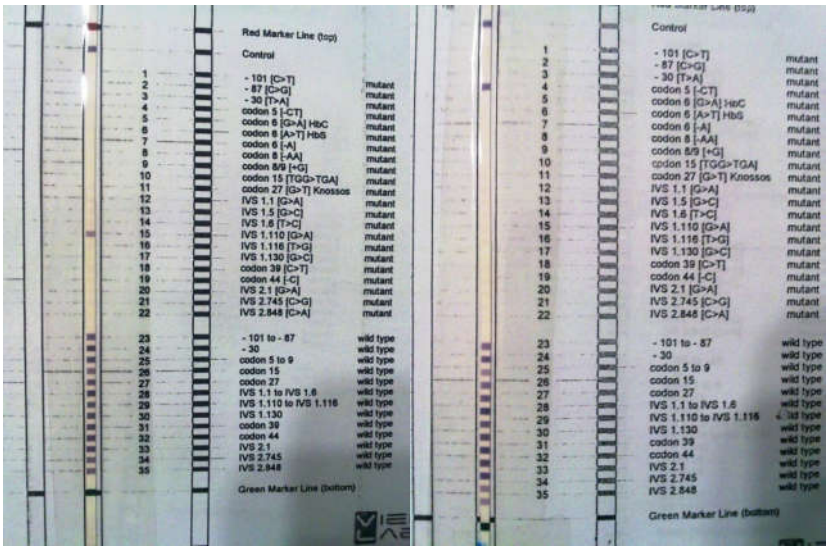


Figure 1: β thalassemia mutations frequency in carriers



[DOI: 10.18502/ijpho.v9i3.1163]

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

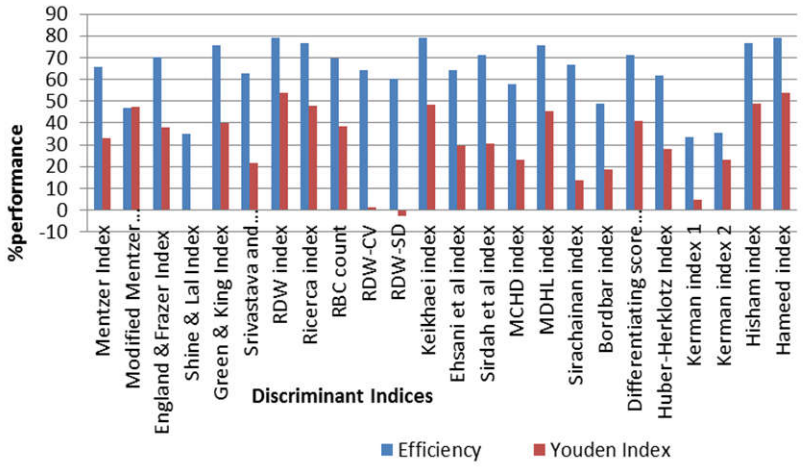


Figure 3: Diagnostic Performance of The Studied Discriminant Indices

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 βTT 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 βTT than IDA. Meanwhile, MCV and RDW were significantly lower in βTT than IDA. Reduction of MCV and MCH values in βTT 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 βTT. Then, the twenty four indices were retrospectively calculated for all cases and each index was evaluated for its discriminating power between IDA and βTT 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 βTT 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

[DOI: 10.18502/ijpho.v9i3.1163]

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 βTT. 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 βTT. 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. Similarily, 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 βTT. In contrast to Melo et al. (44) who proposed that βTT 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 βTT 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

[DOI: 10.18502/ijpho.v9i3.1163]

the diagnosis of βTT. 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 βTT, 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 βTT 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 βTT. 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 βTT or IDA. Studies have shown that these formulas properly identify only 61–91% of the patients with microcytic anemia due to βTT 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

[DOI: 10.18502/ijpho.v9i3.1163]

support and comments that greatly improved the manuscript..

Conflict of interest

Author declared no conflict of interst.

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[DOI: 10.18502/ijpho.v9i3.1163]