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Simple Red Cell Indices in Screening and Discrimination of Iron Deficiency Anemia and Beta Thalassemia Trait in Egyptian Patients

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Abstract

Background: The most common causes of microcytic hypochromic anemia are iron deficiency anemia (IDA) and the beta-thalassemia trait (β-TT). The aim of the work is to compare the validity of the various simple indices to differentiate between iron deficiency anemia and β-thalassemia trait. **Subjects and methods:** A total of 2000 individuals were screened, by complete blood picture, measurement of serum iron, serum ferritin and hemoglobin separation; 224 patients were detected as having hypochromia and microcytosis. Among them 166 cases were IDA and 58 cases were β-TT. We calculated 11 discrimination indices. The number of correctly identified cases were determined, sensitivity, specificity, positive and negative predictive value and Youden's index (YI) of each discrimination index was calculated. **Results:** The percentage of correctly diagnosed patients is highest for Matos and Carvalho index (MCI) (95.5%) which is closely followed by Red cell distribution width index (RDWI) (93.7%). The third high index was Green and King (GKI) (91.9%). Sensitivity, specificity of MCI for detection of IDA was found 98.8%, 87.9% respectively. For β-TT, sensitivity and specificity was found 86.2% and 98.8% respectively. The YI of MCI was found 85.9%. It is followed by RDWI; its sensitivity and specificity for detection of IDA was found 97.6%, 85.3%. For β-TT, sensitivity and specificity was found 82.7% and 97.6% respectively. The YI of RDWI was 81.6%. **Conclusion:** Matos and Carvalho index and RDWI are easily available automated cell-count-based indices coming out as good discriminator between IDA and β-TT in our study.

Keywords

Thalassemia Trait, Iron Deficiency Anemia, RBC Indices

1. Introduction

The most common causes of microcytic hypochromic anemia are iron deficiency anemia (IDA) and the beta-thalassemia trait (β-TT). Beta-Thalassemia is the most common genetically inherited hemoglobin disorder in Egypt [1]. Beta-thalassemia is asymptomatic or clinically manifests as mild anemia and specific hematological characteristics which results from heterozygous mutation of β-globin gene [2]. It is estimated that 1.5% of the world population are beta thalassemia carriers and thalassemia occurring in Egypt at a frequency of 2.1% [3]. Thalassemia screening is difficult, due to heterogeneity of thalassemia and the absence of a pathognomonic finding to cover all variants. So, many attempts have been made to establish screening tests help in differentiation of various forms of microcytic anemia, especially the most common causes, iron deficiency anemia and beta-Thalassemia. Since thalassemia is associated with microcytosis and significant hypochromia, determination of red cell index has been used as a preliminary indication of thalassemia trait [4]. Beta thalassemia carriers considered patients in whom iron deficiency is difficult to exist because dyserythropoiesis enhances iron absorption. However, iron deficiency might coexist or develop in many carriers of beta thalassemia [3]. Microcytosis and hypochromia of red blood cells is a common feature encountered in hemogram of IDA or β-TT patients. However, definitive differential diagnosis between both disorders, especially in countries where both disorders are prevalent is based on the results of more reliable methods like hemoglobin electrophoresis, serum iron levels, serum ferritin and DNA studies for specific mutations. These confirmatory tests could be limited for mass screening programs, due to financial causes, especially in developing and poor countries with restricted resources [4] [5]. The development of indices with good diagnostic accuracy based on parameters derived from the blood cell counters would be useful in the clinical routine [6]. Accurate diagnosis of hypochromic microcytic anemia is an everyday concern for physicians; for appropriate treatment and prevention of disease [7]. The aim of the work is to compare the validity of the various simple indices to differentiate between iron deficiency anemia and β-thalassemia trait.

2. Subjects and Methods

2.1. Subjects and Study Design

The purpose of the study is screening for iron deficiency anemia and beta-thalassemia trait among patient attending the outpatient clinic at Sohag university hospital for investigation or couples intending to marry, in 2011. All of the individuals were 2000 in number, 224 patients were detected as having hypochromia and microcytosis in their red blood cells (RBCs) in whom mean corpuscular volume (MCV) <76 fl and/or mean corpuscular hemoglobin (MCH) <27 pg. Among them 166 cases were IDA and 58 cases were β-TT. A definitive differential diagnosis between IDA and β-TT is based on the results of serum iron levels, serum ferritin and hemoglobin separation. Iron deficiency anemia

was based on low serum iron and serum ferritin (<30 μg/dl, <12 ng/ml respectively). While the diagnosis of thalassemia trait was recognized by HbA_2 > 3.5% and <8%. Informed consent was obtained from the patients.

Exclusion criteria were: mixed cases of IDA and β-TT, microcytic hypochromic cases with normal iron profile and normal hemoglobin electrophoresis, inflammatory diseases, infections, hypothyroiditis, acute bleeding, or any kind of malignancies that might affect ferritin or haemoglobin levels. The study was approved by the committee of research ethics in our faculty.

2.2. Methods

10-ml venous blood was obtained from each subject, in the appropriate vacutainers provided. The K2-EDTA tubes were for complete blood picture and HBA_2 estimation. Complete blood picture to assess red blood cell count, haemoglobin (Hb), haematocrit (HCT), mean corpuscular volume (MCV), and mean corpuscular haemoglobin (MCH) and red cell distribution width (RDW). Complete blood picture was done on cell Dyne-3700 fully automated cell counters, Abbot Diagnostics, German. Separation of Hb was determined by ion-exchange high-performance liquid chromatography (HPLC) (The BioRad D10 analyser, United States). The D-10 Dual Program is based on chromatographic separation of Hb on a cation exchange cartridge. The samples are automatically diluted and injected into the analytical cartridge. The D-10 delivers a programmed buffer gradient of increasing ionic strength to the cartridge, where the hemoglobins are separated based on their ionic interactions with the cartridge material. The separated hemoglobins then pass through the flow cell of the filter photometer, where changes in the absorbance at 415 nm are measured. Serum iron was determined by Cobas 311, fully automated chemical auto-analyzer (Roche/Hitachi cobas c systems, Japan). Under acidic condition, iron is liberated from transferrin. A scorbate reduce Fe^{3+} ions to Fe^{2+} ions which then react with Ferrozine to form colored complex measured photometrically. Serum ferritin test was performed using fully automated Architect instrument (Abbott Diagnostics Division, Chicago) based on Chemiluminescent microparticle immunoassay (CMIA). Ferritin assay is a two-step immunoassay. In the first step, sample containing ferritin and anti-ferritin coated paramagnetic microparticles are combined. After washing, anti-ferritin acridinium labeled conjugate is added in the second step. Pre-trigger and trigger Solutions are then added to the reaction mixture; the resulting chemiluminescent reaction is measured as relative light units (RLUs). A direct relationship exists between the amount of ferritin in the sample and the RLUs detected by its optical system.

The following cell counter based formulas were applied in each case. The differential threshold value for each discrimination indices was demonstrated in Table 1:

1) Mentzer index (MI) = MCV/RBC [8].

2) Shine and Lal index (SLI) = MCV × MCV × MCH/100 [9].

Table 1. Threshold values of the indices used to discriminate IDA and β-TT.

Indices	IDA	β-TT
RBC count	<5	> 5
RDW	>14	<14
MI	>13	<13
SLI	>1530	<1530
EFI	Positive (>0)	Negative (<0)
SI	>3.8	<3.8
GKI	>72	<72
RDWI	>220	<220
RI	>4.4	<4.4
EI	>15	<15
SehI	>972	<972
HHI	>20	<20
MCI	<23.85	>23.85

3) England and Fraser index (EFI) = MCV − v RBC − 5Hb [10].
4) Srivastava index (SI) = MCH/RBC [11].
5) Green and King index (GKI) = MCV × MCV × RDW/(Hb × 100) [12].
6) Red cell distribution width index (RDWI) = MCV × RDW/RBC [13].
7) Ricerca index (RI) = RDW/RBC [14].
8) Ehsani index (EI) = MCV − 10 × RBC [15].
9) Sehgal index (SehI) = MCV × MCV/RBC [16].
10) Huber-Herklotz index (HHI) = MCV × RDW × 0.1/RBC + RDW [17].
11) Matos and Carvalho index (MCI) = 1.91 × RBC + 0.44 × MCHC [6].

2.3. Statistical Analysis

Statistical analyses were performed using SPSS version 19. Data were summarized as means ± SD, percentage and range. The differences between groups were compared by T-test. The indices data were analyzed using Microsoft office excel. The validity of discrimination indices were evaluated by calculating their sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and Youden's index (YI). Statistical significance is considered a value of $P < 0.05$.

3. Results

The baseline demographic and laboratory variables of the studied groups were demonstrated in Table 2. A total 224 patients their age were ranged from 18 to 40 years. Their age was a mean 30.3 ± 5.6 for IDA patients (72 males and 94 females). Beta-thalassemia trait patient's age was a mean 23.4 ± 4.1 (38 males and 20 females). Beta-thalassemia trait patients showed significantly higher mean values of RBCs, Hb, HCT and MCHC, but significantly lower mean values

Table 2. Baseline demographic and laboratory characteristics of the study groups.

Variables	IDA (N = 166) Mean ± SD	β-TT (N = 58) Mean ± SD	P
Age	30.3 ± 5.6	23.4 ± 4.1	
Male: Female %	0.8 - 1	1.9 - 1	
S. iron µg/dl	21 ± 8.3	78 ± 20.3	<0.0001
S. feritin ng/ml	8 ± 3.2	38 ± 12.3	<0.0001
HbA_2 %	2.3 ± 0.5	5.3 ± 0.89	<0.0001
RBCs × 10^{12}/l	4.7 ± 0.38	5.6 ± 0.57	0.003
Hb g/dl	9.9 ± 1.01	11.9 ± 2.3	0.03
HCT %	33.1 ± 2.4	37.9 ± 6.8	<0.0001
MCV fl	70.04 ± 6.45	62.6 ± 8.3	0.03
MCH pg	21.7 ± 6.6	20.2 ± 3.3	0.01
MCHC g/dl	30.02 ± 1.5	32.2 ± 2.06	0.01
RDW%	18.6 ± 2	18.6 ± 3.4	0.0002

MCV, MCH and RDW than IDA patients. The number and percentage of correctly diagnosed patients' calculation are shown in Table 3. This table reveals that percentage of correctly diagnosed patients is highest for MCI (95.5%) which is closely followed by RDWI (93.7%). The third high index was GKI (91.9%). This followed by, EFI (89.2%) then MI (82.1%) then SI (80.4%). Thus according to percentage correctly diagnosed criteria MCI, RDWI and GKI are considered as the three best discrimination indices. The Sensitivity, Specificity, Positive predictive value, Negative predictive value and the YI of each discrimination index in differentiating between IDA and β-TT groups are given in Table 4. Analysis of this table indicates that, MCI came out as good discriminators between IDA and β-TT. Sensitivity, specificity of MCI for detection of IDA was found 98.8%, 87.9% respectively. For β-TT, sensitivity and specificity was found 86.2% and 98.8% respectively. The YI of MCI was found 85.9. It is followed by RDWI, its sensitivity and specificity for detection of IDA was found 97.6%, 85.3%. For β-TT, sensitivity and specificity was found 82.7% and 97.6% respectively. The YI of RDWI was 81.6. The third one was GKI which has sensitivity, specificity for IDA cases were found, 98.8%, and 76.3% respectively and for β-TT sensitivity was 72.4% and specificity was 98.8%. Ultimate YI was found 73.2.

4. Discussion

Many attempts are done in the last decades to simplify the differential diagnosis between IDA and β-TT, and as a result, several indices using blood cell count parameters are made but none of these indices or formulas provided an absolute discrimination power. The index or formula that offered the highest discrimination power for one population, yet, it showed moderate or low power when applied to other populations [5]. Application of these indices depends on racial

Table 3. Correctly identified number and percentage of patients.

Indices	IDA (N = 166)	β-TT (N = 58)	Total correctly identified	Percentage
RBCs count				
IDA	120	8	170	75.8%
β-TT	46	50		
RDW				
IDA	164	56	166	74.1%
β-TT	2	2		
MI				
IDA	136	10	184	82.1%
β-TT	30	48		
SLI				
IDA	26	8	76	33.9%
β-TT	140	50		
EFI				
IDA	166	24	200	89.2%
β-TT	-	34		
SI				
IDA	130	8	180	80.4%
β-TT	36	50		
GKI				
IDA	164	16	206	91.9%
β-TT	2	42		
RDWI				
IDA	162	10	210	93.7%
β-TT	4	48		
RI				
IDA	16	4	70	31.3%
β-TT	150	54		
EI				
IDA	130	10	178	79.5%
β-TT	36	48		
SehI				
IDA	86	8	93	41.5%
β-TT	80	50		
HHI				
IDA	166	56	168	75%
β-TT	0	2		
MCI				
IDA	164	8	214	95.5%
β-TT	2	50		

differences; furthermore, the degree of the active hematopoiesis fluctuates in different growth periods, and consequently, affecting the levels of hematological parameters. Therefore, the applicability of these hematological indices might exhibit a slight difference in various populations [18]. So we aimed to compare various simple indices in our population.

In our study, the percentage of correctly diagnosed patients, is highest for MCI and RDWI 95.5% and 93.7%, respectively, followed by GKI 91.9%, EFI

Table 4. Sensitivity, specificity, PPV, NPV and YI of discrimination indices in diagnosis of IDA and *β*-TT.

Indices	Sensitivity %	Specificity %	PPV %	NPV %	Youden's Index
RBCs count					
IDA	72.3	87.8	88.2	55.8	62.3
β-TT	86.2	78.3	52.1	95.4	
RDW					
IDA	98.8	50.8	74.5	96.7	25.9
β-TT	3.4	98.8	50	74.8	
MI					
IDA	81.9	85.3	93.2	65.9	67.3
β-TT	82.7	84.7	61.5	94.3	
SLI					
IDA	15.7	87.9	76.5	29.3	44
β-TT	86.2	54.2	26.3	95.4	
EFI					
IDA	100	70.7	87.4	100	64.7
β-TT	58.6	100	100	87.4	
SI					
IDA	78.3	87.9	94.2	61.7	67.3
β-TT	86.2	82.2	58.1	95.4	
GKI					
IDA	98.8	76.3	91.1	96.7	73.2
β-TT	72.4	98.8	95.5	91.2	
RDWI					
IDA	97.6	85.3	94.2	98.3	81.6
β-TT	82.7	97.6	92.3	94.3	
RI					
IDA	9.6	93.5	80	27.9	48.7
β-TT	93.1	52.5	26.5	97.6	
EI					
IDA	78.3	85.3	92.8	61.7	64.3
β-TT	82.8	82.2	57.1	94.3	
SehI					
IDA	51.8	87.9	91.5	42	46.7
β-TT	86.2	67.5	38.5	95.4	
HHI					
IDA	100	50.9	74.8	74.8	27.2
β-TT	3.4	100	100	100	
MCI					
IDA	98.8	87.9	95.3	96.7	85.9
β-TT	86.2	98.8	96.2	95.4	

89.2%, MI 82.1%, SI 80.4% then EI, RBCs, HHI and RDW but SehI, SLI and RI showed lowest percentage. Matos *et al.*, [6] introduce a new formula in 2016 and this index gives a high proportion of individuals correctly identified with the disease. A similar result for RDWI was reported by other relevant studies [3]

[19] [20]. On the other hand Vehapoglu *et al.*, [21] concluded that the percentage of correctly diagnosed patients were the highest with the Mentzer index (91%) followed by the Ehsani index (84.8%) and RBC count (83.4%).

Sensitivity and specificity are very important parameters required by a screening test to be validated. Diagnostic ability of a test method highly depends on these parameters [22]. In this study, we observed that none of the discrimination index showed 100% sensitivity and specificity. Higher sensitivity and specificity of indices for IDA and β-TT were observed in MCI, RDWI and GKI. The sensitivity and specificity of MCI in the detection of IDA were found 98.8% and 87.9%, respectively, and the sensitivity and specificity for the detection of β-TT were 86.2% and 98.8%, respectively. The highest YI was obtained for MCI (85.9%). The Youden's index considers both sensitivity and specificity and provides an appropriate measure of the overall validity of the index or technique [23]. Matos *et al.*, [6] reported that its sensitivity was 99.3%, Specificity 76.7% and YI was 76.0%, and the evaluation of this index by Arora *et al.*, [22] showed high sensitivity to detect IDA (98.81%), excellent diagnostic accuracy and showing good applicability as screening tool in clinical practice. On the other hand Sirdah *et al.*, [5] finding was different, sensitivity & specificity were 67.0% and 72.3% respectively, and YI was (39.3%). The sensitivity and specificity of RDWI in the detection of IDA were found 97.6% and 85.3%, respectively and the sensitivity and specificity for the detection of β-TT were 82.7% and 97.6%, respectively. The RDWI had high YI (81.6%). These results are consistent with the findings of other relevant studies who found that the highest YI was obtained for RDWI 69.2%, 83% and 73.8% respectively [23] [24] [25]. The present study found the MCI and RDWI indices to be best at differentiating IDA from β-TT. On the other hand okan *et al.*, [7] found that RDWI to be the worst index in differentiating IDA from β-TT. The YI of GKI is (73.2%), YI of MI and SI is equal (67.3%), followed by, EFI, EL and RBCs. Huang *et al.*, [26] reported that RDWI and GKI exhibited improved sensitivity IDA patient's relative to either α-TT or β-TT patients.

Our study showed that RDW formula is the lowest value for YI, suggesting that this formula is least effective in discrimination studies for microcytic anemia. Although, the RDW is a valued, discrimination index for differentiating β-TT and IDA [27], our results found that RDW is almost equally elevated in both β-TT and IDA. The mean values of RDW, found in IDA and β-TT were 18.6 (SD ± 2) and 18.6 (SD ± 3.4) respectively. Its YI was found 25.9, which would not be a good discriminator of IDA and β-TT in our population. Similar findings also reported by other studies [28] [29].

Limitation of the study: This present study has a relative small sized sample; larger sample size is needed in the future study. Unfortunately the DNA analysis and genetic variation couldn't be included in our study group of β-TT, and the same for α-TT detection. Sex-based indices and formulas are necessary to improve the reliability in mathematically discriminating between IDA and β-TT in

mass screening programs.

5. Conclusion

Matos and Carvalho index and RDWI are easily available automated cell count-based indices coming out as good discriminator between IDA and β-TT in our study; its sensitivity and specificity were more than 80% in detection of β-TT and IDA. Both indices are appearing to be reliable and could be useful in initial screening of microcytic hypochromic anemia because its high sensitivity and PPV which may result in a significant cost saving for the health system and advantageous in developing countries with limited financial resources.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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