Med. J. Cairo Univ., Vol. 86, No. 7, December: 3979-3988, 2018
www.medicaljournalofcairouniversity.net

The Diagnostic Performance of Red Cell Distribution Width and Mentzer Index for Discrimination between Iron Deficiency Anemia and Beta Thalassemia Trait

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Abstract

Background: Mild microcytic hypochromic anemias due to Iron Deficiency (IDA) and Beta Thalassemia Trait (β-TT) continue to be a cause of significant burden to the society, particularly in the poorer developing countries, as β-TT is often misdiagnosed as iron deficiency anemia in children because the two produce similar hematologic abnormalities on Complete Blood Count (CBC), and IDA is much more prevalent. So it is important to differentiate between thalassemic and non thalassemic microcytosis as both conditions share many characteristics and have important clinical implications. Thus a correct diagnosis in patients with microcytic anemia can provide an indication for supplementing iron to IDA patients, for avoiding unnecessary iron therapy in thalassemia carriers and of course also for preventing severe and lethal forms of thalassemia syndromes in the framework of premarital counseling in high-prevalence areas.

Aim of Study: To evaluate the diagnostic performance of simple and costless methods, i.e. red cell distribution width (RDW%), red cell distribution width index (RDWI) and Mentzer index for discrimination between IDA and β-TT.

Patients and Methods: The study was conducted on 50 patients with microcytic hypochromic anemia, 26 males and 24 females aged from 2- 4 years recruited from the Hematology Outpatient Clinic. In addition, 15 apparently healthy children with matchable age and sex, selected from relatives of the patients were enrolled in the study as a control group.

Results: The highest sensitivity rate (100%) was shown by RDW index but with a low specificity rate, (79.3 1%) followed by Mentzer as well as Green and King indices with a sensitivity=95.24% for each, while the specificity rate of Mentzer index was 93. 1% and that of Green and King index was 79.31%.

Conclusion: Among the studied indices, reduced Mentzer index <13 showed the highest diagnostic performance for discrimination between iron deficiency anemia and beta thalassemia trait by detection of increased Hb A2 >3.5% as proved by ROC test.

Recommendation: As discrimination between iron deficiency anemia and beta thalassemia trait is very essential to reach the suitable therapeutic strategy for such cases, therefore, we recommend the use of Mentzer index as a feasible, costless method with a high diagnostic performance for preliminary discrimination between those two diseases.

Key Words: *Beta Thalassemia Trait – Iron Deficiency Anemia – Mentzer index – RDW% – RDW index.*

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Introduction

MILD microcytic hypochromic anemias due to Iron Deficiency (IDA) and Beta Thalassemia Trait (β-TT) continue to be a cause of significant burden to the society, particularly in the poorer developing countries, as β-TT is often misdiagnosed as iron deficiency anemia in children because the two produce similar hematologic abnormalities on Complete Blood Count (CBC), and IDA is much more prevalent. So it is important to differentiate between thalassemic and non thalassemic microcytosis as both conditions share many characteristics and have important clinical implications. Thus a correct diagnosis in patients with microcytic anemia can provide an indication for supplementing iron to IDA patients, for avoiding unnecessary iron therapy in thalassemia carriers and of course also for preventing severe and lethal forms of thalassemia syndromes in the framework of premarital counseling in high-prevalence areas [1].

Iron Deficiency Anemia (IDA) and beta thalassemia trait (β-TT) are the two most frequent disorders presenting clinically with mild microcytic hypochromic anemia [2]. Lack of sufficient dietary iron resulting in IDA is the most common hematological disorder. It has been estimated that 30% of the world population suffers from IDA with majority of the affected people living in developing

countries. In β-TT there is impaired globin chain synthesis resulting in decreased hemoglobin leading to microcytic hypochromic anemia. 1.5% of world population carries genes for beta thalassemia [3]. Thalassemia traditionally has a high prevalence in some parts of the world (Mediterranean regions up to 8%; countries of the Middle East up to 10%; India 3%-15%; Southeast Asia up to 9%), where it represents a major public health problem. Non endemic countries such as in Northern Europe and North America are also involved in thalassemia related problems as a result of demographic changes caused by migration and intermarriages of different ethnic populations [4,5]. Nowadays population migration has spread thalassemia genes over nearly the entire globe [6], so it is important to differentiate between thalassemic and non thalassemic microcytosis as both conditions share many characteristics and have important clinical implications [8]. Thus a correct diagnosis in patients with microcytic anemia can provide an indication for supplementing iron to IDA patients, for avoiding unnecessary iron therapy in thalassemia carriers and of course also for preventing severe and lethal forms of thalassemia syndromes in the framework of premarital counseling in high-prevalence areas [1]. A definitive differential diagnosis between β-TT and IDA is based on the result of HBA_2 electrophoresis, serum iron levels, and ferritin calculations [9]. However, these investigations are money and time consuming and moreover the areas where thalassemia is endemic often have low health care resources and these assays may not be generally available [1]. Thus, various discrimination indices have been proposed to distinguish between β-TT and IDA. These indices are derived from several simple Red Blood Cell (RBCs) indices, like RBCs count, Mean Cell Volume (MCV), mean corpuscular hemoglobin (MCH), RBCs Distribution Width (RDW) and Hemoglobin (Hb) as these are provided by electronic cell counters [10]. The purpose of using indices to discriminate anemia is to detect subjects who have a high probability of requiring appropriate follow-up and to reduce unnecessary investigative costs [11]. An ideal discrimination index has high sensitivity and specificity; that is, it can detect the maximum number of patients with β-TT (high sensitivity) while eliminating patients with IDA (high specificity) [11].

Subjects and Methods

The study was conducted on 50 patients with microcytic hypochromic anemia, 26 males and 24 females aged from 2-4 years recruited from the Hematology Outpatient Clinic, Assuit University Children Hospital, during the period from the 1st of June, 2016 to the 30th of May, 2017. In addition, 15 apparently healthy children with matchable age and sex, selected from relatives of the patients were enrolled in the study as a control group.

Inclusion criteria:

Microcytic hypochromic anemia with hemoglobin level not less than 7g/dl.

Exclusion criteria:

- Hemoglobin level less than 7g/dl.
- Chronic disease or infection.
- Lead poisoning.
- History of previous blood transfusion.

Beside meticulous history and thorough clinical examination. All the studied cases and controls were subjected to the following investigations Complete Blood Count (CBC), Blood film, Serum iron, Serum ferritin, Total Iron Binding Capacity (TIBC) and hemoglobin electrophoresis, and the other discrimination indices were calculated by using RBCs indices as defined below:

- *Mentzer Index (MI): MCV/RBC* [12]:

Interpretation:

IDA: >13.

BTT: <13.

- *Green and King Index (G & K): MCV2 X RDW/ 100 X Hb* [13]:

Interpretation:

IDA: >72.

BTT: <72.

- *RDW Index (RDWI): MCV X RDW/RBC* [14]:

Interpretation:

IDA: >220.

BTT: <220.

- *Evaluation of red cell distribution width (RDW):*

Red cell distribution width quantitatively measures RBCs size variation, computed directly from the RBCs histogram and is calculated as a standard statistical value, the coefficient of variation of the volume distribution [15]. In general, an elevated RDW has been associated with anemia from various deficiencies such B12, folate or iron [16]. So RDW as a measure of the degree of variation in red cell size, has been reported to be a good discrimination index to differentiate between β-TT and IDA [17].

Interpretation:

IDA: >14.

BTT: <14.

The sensitivity and specificity, and positive and negative predictive values were calculated as follows: Sensitivity: True positive/(true positive + false negative); specificity: True negative/(true negative + false positive); positive predictive value: True positive/(true positive + false positive); negative predictive value: (True negative/true negative + false negative).

Statistical analysis:

The data were coded and verified prior to data entry. The Statistical Package for Social Science (SPSS) Version 19 (Microsofot Windows, SPSS Inc), was used for data entry and analysis. Data were checked for Gaussian distribution by the Shapiro-Wilk statistic. Parameters with Gaussian distribution were expressed as mean ± SD; between group differences they were assessed by independent samples *t*-test for parametric data and Mann-Whitney test for non parametric data. The Chi-square test (χ^2) was used to compare the frequency of qualitative variables among the studied groups **[18]**.

A p-value >0.05 was determined as non significant, while p-value <0.05 was considered significant **[18]**.

The diagnostic performance of RBCs count, Hb level, HCT value, reticulocytic count, MCV, MCH, MCHC, RDW%, serum iron, serum ferritin and TIBC as well as Mentzer, RDW, Green and King Indices for detection of increased Hb A2 serum level (>3.5%) among cases with hypochromic microcytic anemia and control group was evaluated using Receiver Operating Characteristic (ROC) curve analysis. The optimal cut off values was determined by Youden index **[18]**.

Results

The majority of patients in the present study had no obvious complaints related to anemia but weakness followed by fatigue was the next common complaints received. On the basis of HBA_2 levels, 21 patients having HBA_2 more than 3.5% were grouped into β-TT group. The rest 29 patients having HBA_2 less than 3.5% were included in the IDA group.

Table (1) shows significant increase of RBCs count and reticulocytic count among cases with BTT (5.02±0.42 and 4.89±2.07 respectively) compared to cases with iron deficiency anemia (4.32 ± 0.44 and 1.75± 1.45 respectively) (p<0.001 for each), significant increase of hemoglobin concentration and hematocrite value among cases with BTT (9.27± 1.05 and 29.18±3.44 respectively) compared to cases with iron deficiency anemia (8.53±0.98 and 27.55±3.59 respectively) (p<0.031 for each). Also, significant increase of RBCs count and reticulocytic count among cases with BTT (5.02±0.42 and 4.89±2.07 respectively) compared to controls (4.74±0.27 and 1.19±0.56 respectively) (p<0.031 and p<0.001 respectively), while significant decrease of hemoglobin concentration and hematocrite value were found among cases with BTT (9.27± 1.05 and 29.18±3.44 respectively) compared to controls (12.19 ±0.47 and 37.41 ± 1.17 respectively) (p<0.001 for each). In addition, significant decrease of RBCs count, hemoglobin concentration and hematocrite value were found among cases with iron deficiency anemia (4.32 ±0.44, 8.53±0.98 and 27.55±3.59 respectively) compared to controls (4.74±0.27, 12.19±0.47 and 37.41 ± 1.17 respectively) (p<0.001 for each).

Also it shoos significant decrease of MCV and red cell distribution width among cases with BTT (58.45±3.81 and 13.42±2.19 respectively) compared to cases with iron deficiency anemia (63.41 ± 4.74 and 18.37±3.37 respectively) (p<0.001 for each), while significant decrease of MCV, MCH and MCHC were found among cases with BTT (58.45±3.81, 19.26±2.87 and 30.20±2.48 respectively) compared to controls (79.68 ±4.69, 28.13± 2.41 and 34.66± 1.75 respectively) (p<0.001 for each). Also, significant decrease of MCV, MCH and MCHC were found among cases with IDA (63.41 ±4.74, 20.09±3.33 and 30.58±2.09 respectively) compared to controls (79.68 ±4.69, 28.13± 2.41 and 34.66± 1.75 respectively) (p<0.001 for each), while significant increase of red cell distribution width of RBCs was found among cases with IDA (18.37±3.37) compared to controls (13.33 ± 0.73) (p<0.001).

Cases with BTT showed significant increase of serum iron and serum ferritin (60.97 ±31.09 and 140.09± 111.57 respectively) compared to cases with IDA (19.27±5.70 and 13.05±6.50 respectively) (p<0.001 for each), while significant decrease of TIBC value was found among cases with BTT (287.76±71.62) compared to those with IDA (497.55±60.29) (p<0.001). Also, significant decrease of serum iron and serum ferritin were found among cases with IDA (19.27±5.70 and 13.05±6.50 respectively) compared to controls (74.20 ±22.38 and71.13±26.36 respectively) (p<0.001 for each), additionally, significant increase of TIBC value was found among cases with IDA (497.55 ±60.29) compared to controls (271.27 ±41.09) (p<0.001) as in (Table 1).

Table (1): Mean values and standard deviation of various hematological and biochemical parameters.

The studied parameters	I BTT (n=21) Mean ± SD	II IDA (n=29) Mean ± SD	III Control group (n=15) Mean ± SD	Significance of difference		
				I vs. II	I vs. III	II vs. III
RBCs (X 10^6/µl):						
Mean ± SD	5.02±0.42	4.32±0.44	4.74±0.27	0.001	0.031	0.001
Range	4.3-6.1	3.3-5.2	4.4-5.3			
HB (g/dl):						
Mean ± SD	9.27±1.05	8.53±0.98	12.19±0.47	0.031	0.001	0.001
Range	7.0-11.6	7.0-10.5	11.4-13.0			
HCT (%):						
Mean ± SD	29.18±3.44	27.55±3.59	37.41±1.17	0.031	0.001	0.001
Range	21.0-34.2	17.6-34.1	34.5-38.8			
Reticulocytic count (%):						
Mean ± SD	4.89±2.07	1.75±1.45	1.19±0.56	0.001	0.001	0.242
Range	0.2-8.5	0.2-2.5	0.4-2.2			
MCV (fL):						
Mean ± SD	58.45±3.81	63.41±4.74	79.68±4.69	0.001	0.001	0.001
Range	46.1-64.0	53.9-69.7	72.8-93.0			
MCH (pg):						
Mean ± SD	19.26±2.87	20.09±3.33	28.13±2.41	0.355	0.001	0.001
Range	13.0-24.7	15.2-31.7	23.4-33.0			
MCHC (g/dl):						
Mean ± SD	30.20±2.48	30.58±2.09	34.66±1.75	0.687	0.001	0.001
Range	25.4-33.9	26.3-35.4	29.6-37.0			
Red cell distribution width (%):						
Mean ± SD	13.42±2.19	18.37±3.37	13.33±0.73	0.001	0.574	0.001
Range	10.4-18.3	14.2-28.5	12.0-14.3			
Serum iron (µg/dl):						
Mean ± SD	60.97±31.09	19.27±5.70	74.20±22.38	0.001	0.180	0.001
Range	15.0-116.0	11.0-31.0	45.0-118.0			
Serum ferritin (ng/ml):						
Mean ± SD	140.09±111.57	13.05±6.50	71.13±26.36	0.001	0.188	0.001
Range	5.2-350.0	4.1-28.0	35.0-126.0			
TIBC (µg/dl):						
Mean ± SD	287.76±71.62	497.55±60.29	271.27±41.09	0.001	0.809	0.001
Range	196.0-490.0	418.0-750.0	196.0-380.0			

Table (2) shows significant decrease of Mentzer index, RDW index as well as Green and King index among cases with BTT (11.73±1.27, 156.08±22.10 and 49.63±8.45 respectively) compared to cases with IDA (14.85±2.02, 273.85±71.16 and 88.03±24.18 respectively) (p<0.001 for each).

Table (2): Values of the studied diagnostic indices in relation to diagnosis of the studied cases.

	BTT (n=21)	IDA (n=29)	Significance of difference
Mentzer index:			
Mean ± SD	11.73±1.27	14.85±2.02	0.001
Range	8.0-13.0	10.8-18.6	
RDW index:			
Mean ± SD	156.08±22.10	273.85±71.16	0.001
Range	122.9-209.0	188.4-481.3	
Green and king index:			
Mean ± SD	49.63±8.45	88.03±24.18	0.001
Range	36.7-73.1	59.4-164.4	

As regard the predictive values of the studied parameters for detection of increased HbA 2 (>3.5%) Table (3) shows:

- The highest sensitivity rate (100%) was shown by RDW index but with a low specificity rate, (79.31 %) followed by Mentzer as well as Green and King indices with a sensitivity=95.24% for each, while the specificity rate of Mentzer index was 93.1 % and that of Green and King index was 79.31%.

- The highest specificity rate (100%) was showed by each of RDW%, serum ferritin, serum iron as well as TIBC which showed the highest sensitivity rate among these parameters 90.48%.

- As regards the +ve and –ve predictive values; RDW%, serum iron, serum ferritin and TIBC showed the highest positive predictive value in predicting increased HbA 2 (>3.5%) (100% for each), but the highest negative predictive value among these parameters was shown by TIBC (93.5%). About the negative predictive value, the highest value among all the studied parameters was shown by RDW index (100%), followed by Mentzer, Green and King indices and TIBC (96.4%, 95.8% and 93.5% respectively).

- A ROC test was done to evaluate the diagnostic performance of the studied parameters for detection of increased Hb A2 (>3.5%) among cases with microcytic hypochromic anemia and control group (Table 3) and Figs. (1-3), it was found that the optimal cut of values of the studied parameters were >4.57 X 10^6 / µl for RBCs, >8.8g/dl for hemoglobin, >28.9% for HCT, >2.9% for reticulocytic count, ≤61fL for MCV, ≤20.5g/dl for MCH, ≤29g/dl for MCHC, <14% for RDW, >31 µg/dl for serum iron, >28 µg/l for ferritin, ≤350 µg/dl for TIBC, <13 for Mentzer index, <220 for RDW index and <72 for Green and King index.

- Total iron binding capacity showed the highest value of AUC (0.973) among the studied parameters with 95% CI 0.882-0.998, followed by serum iron, serum ferritin and Mentzer index (0.954, 0.943 and 0.942 respectively) with 95% CI (0.854-0.993, 0.839-0.989 and 0.837-0.988 respectively).

- Concerning the diagnostic accuracy (ACC) for detection of increased HbA 2 (>3.5%), TIBC showed the highest ACC (96%) with 95% CI (92.7-99.3) followed by serum iron, serum ferritin and Mentzer index (94% for each) with 95% CI (91-97, 91.8-96.2 and 91.5-96.2 respectively).

Table (3): Diagnostic performance of the studied parameters and indices for detection of increased HbA 2 serum level (>3.5%) among cases with microcytic hypochromic anemia and control group.

The studied parameters and indices	Optimal cut of value	Sensitivity %	Specificity %	PPV %	NPV %	AUC	95% CI of AUC	ACU %	95% CI of ACC
The parameters:									
RBC (10^6/µl)	>4.57	90.48	79.31	76.0	92.0	0.895	0.775-0.964	84.00	81.9-86.1
Hb (g/dl)	>8.8	71.43	72.41	65.2	77.8	0.680	0.533-0.805	72.00	71.3-73.7
HCT (%)	>28.9	71.43	72.41	65.2	77.8	0.680	0.533-0.805	72.00	70.5-73.5
RET (%)	>2.9	90.48	89.66	86.4	92.9	0.903	0.786-0.969	90.00	86.3-93.2
MCV (fL)	≤61	85.71	72.41	69.2	87.5	0.782	0.643-0.887	78.00	76.5-79.5
MCH (pg)	≤20.5	80.95	51.72	54.8	78.9	0.577	0.429-0.716	64.00	62.8-65.2
MCHC (g/dl)	≤29	47.62	79.31	62.5	67.6	0.534	0.387-0.676	66.00	64.5-67.5
RDW (%)	<14	71.43	100.00	100.0	82.9	0.857	0.729-0.940	88.00	85.9-90.1
Serum Iron (µg/dl)	>31	85.71	100.00	100.0	90.6	0.954	0.854-0.993	94.00	91-97
Serum Ferritin (µg/l)	>28	85.71	100.00	100.0	90.6	0.943	0.839-0.989	94.00	91.8-96.2
TIBC (µg/dl)	≤350	90.48	100.00	100.0	93.5	0.973	0.882-0.998	96.00	92.7-99.3
The indices:									
Mentzer index	<13	95.24	93.10	90.9	96.4	0.942	0.837-.988	94.00	91.5-96.2
RDW index	<220	100.00	79.31	77.8	100.0	0.897	0.778-0.965	88.00	83.7-90.3
Green and King index	<72	95.24	79.31	76.9	95.8	0.873	0.748-0.950	86.00	83.7-88.3

AUC : Area Under Curve.
ACC : Accuracy.
PPV : Positive Predictive Value.
NPV : Negative Predictive Value.
95% CI : 95% confidence interval.

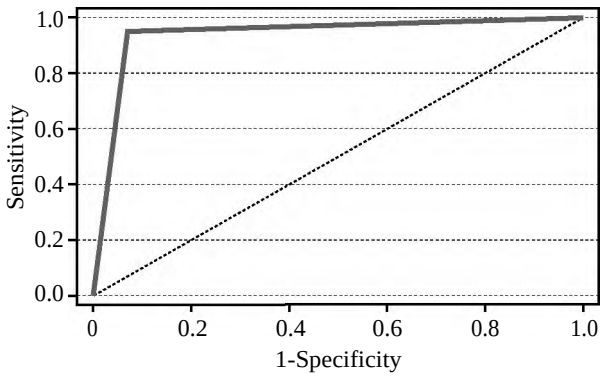

Fig. (1): Receiver Operating Characteristic (ROC) curve to determine the diagnostic performance of Mentzer index for detection of increased Hb A2 serum level (>3.5%) among the studied caseswith hypochromic microcytic anemia and control group.

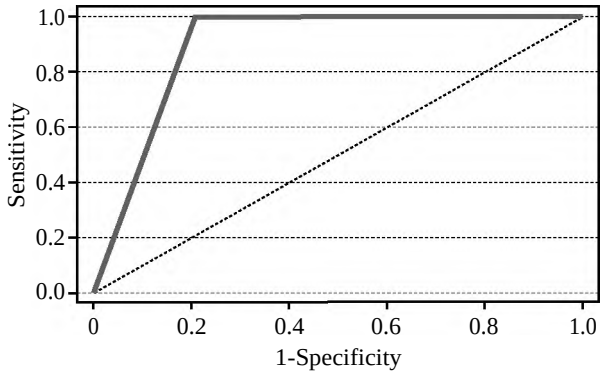

Fig. (2): Receiver Operating Characteristic (ROC) curve to determine the diagnostic performance of RDW index for detection of increased HbA_2 serum level (>3.5%) among the studied caseswith hypochromic microcytic anemia and control group.

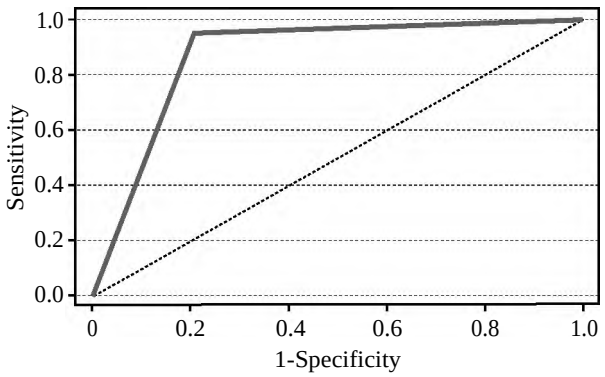

Fig. (3): Receiver Operating Characteristic (ROC) curve to determine the diagnostic performance of Green and King index for detections of increased HbA_2 serum level (>3.5%) among the studied caseswith hypochromic microcytic anemia and control group.

Discussion

In the present study, patient with BTT showed significant increase in RBCs count (5.02 ±0.42 X 10^6/ µL) compared to those with IDA (4.32±0.44 X 10^6/ µL) and this was in agreement with Piplani et al., [11] who found that, majority of patients with BTT in their study had a high RBCs count of more than 5 million with mean value (5.79±0.76 X 10^6/ µL) in comparison to IDA (4.77±0.53 X 10^6/ µL). Vehapoglun et al., [20] also stated that, RBCs count was higher in the BTT (5.56±0.4 X 10^6/ µL) group than that in the IDA (4.84±0.59 X 10^6/ µL; p<0.05). Red blood cells count tends to be higher in BTT than in IDA. This increase in RBCs count can be explained by Globin chain precipitation in erythroid precursor cells and circulating erythrocytes that leads to a discrete inefficacious erythropoiesis, causing increased erythrocyte production in an attempt to compensate for anemia (hyper active bone marrow). From these result they concluded that, RBC count alone cannot be taken as a reliable index to distinguish between of BTT and IDA as a high erythrocyte count (RBC >5.0 X 10^6/ µL) is a common feature of IDA and BTT.

In our studied cases there were significant increase of hemoglobin concentration among cases with BTT (9.27±1.05gm/dl) compared to cases with iron deficiency anemia (8.53±0.98gm/dl) this is in agreement with Nesa et al., [21] who found the mean hemoglobin level (10.2±0.8gm/dl) in cases of BTT, and (9.8±0.9gm/dl) in case of IDA, similar results were also found by matos et al., [22] who found significant differences of Hb level between BTT and IDA. On the other hand Piplani et al., and Vehapoglu et al., [11,20] found that the mean values for Hb in BTT were 10.39±0.69 and 10.7+0.8 respectively and those in the IDA group were 10.23±0.95 and 9.9±1.43 respectively (p>0.05) with no significance of differences between them, and they concluded that, hemoglobin count cannot be taken as a reliable index to distinguish between BTT and IDA.

Data of the present study showed significant increase in reticulocytic count among the studied cases with BTT (4.89±2.07%) compared to cases with IDA (1.75±1.45%), and this is in agreement with Noronha and Grotto, [23] who found significant increase in reticulocytic count in patients with BTT than those with IDA. The number of reticulocytes in the peripheral blood is fairly accurate reflection of erythropoietic activity and is an indicator of the erythropoitic activity of bone marrow which is more in patients with BTT.

Our data showed significant decrease of MCV among studied cases with BTT (58.45±3.81fL) compared to studied cases with iron deficiency anemia (63.41±4.74 fL), this is in agreement with the previous studies Piplani et al., [11], Nesa et al., [21], Matos et al., [22] and Ntaios et al., [24] who

found that MCV tend to be lower in BTT compared to IDA with significance of differences <0.05. This was explained by significant increase in RBCs count in BTT than IDA with no significant of difference for HCT among cases with BTT compared to cases with IDA as MCV equal to packed red cell volume divided by RBCs count by millions, and this explains the importance of MCV values as discriminating parameters but using it alone not sufficient to differentiate between BTT and IDA due to low sensitivity and specificity.

Our results show significant decrease of RDW% among cases with BTT (13.42 ±2.19%) compared to those with iron deficiency anemia (18.37 ± 3.37%), with sensitivity 71.43%, specificity 100.00% AUC 0.857 and ACC 88.00%, this is in agreement with Rahim and Keikhaei, **[25]** who found that, significant decrease of RDW% was observed between the groups with BTT (11.79 ± 6.75%) compared to IDA (14.20 ±4.2%) with sensitivity 51%, specificity 87% for BTT and for IDA sensitivity 87% and specificity 51%, Matos et al., **[22]** also found that, significant decrease of RDW% was observed between the groups with BTT (15.9 ± 0.5%) compared to IDA (17.9 ± 1.2%).

Red cell distribution width was first described by Bessman and Feinstein, **[26]** as parameters used for discrimination between BTT and IDA, and nowadays is part of automated counter analyses. Bessman and Feinstein, **[26]** observed that erythrocytes of thalassemia minor patients were more homogeneous than those of iron deficiency anemia patients. Consequently, RDW% tends to be higher in IDA than in BTT **[26]**.

On the other hand Nesa et al., **[21]** found that RDW% more or less equally elevated in BTT and IDA the mean value of RDW%, found in IDA and BTT were 16.9 ±2.9 SD and 16.4 ±2.5 SD respectively with sensitivity 17.5% and specificity 84.7% for BTT, and sensitivity 84.7% and specificity 17.5 for IDA. Similar findings to Nesa et al., **[21]** were also reported by Ntaios et al., **[24]** Flynn et al., **[27]**, Bagar et al., **[28]** and AlFadhli et al., **[29]**, concluded that, a correct discrimination between these disorders could not be done based on just RDW%. Besides those studies, Matos et al., **[22]** did not verify significant difference in RDW% between the three groups of assessed microcytic anemias (IDA, BTT and anemia of chronic disease), indicating that this test has limited usefulness for differentiating iron deficiency anemia, beta thalassemia minor, and anemia of chronic disease **[22]**.

In the present study, the studied cases showed significant increase of serum iron and serum ferritin among the studied cases with BTT (60.97 ±31.0 and 140.09± 111.579 respectively) compared to the studied cases with IDA (19.27 ±5.70 and 13.05 ±6.50 respectively), similar findings were also reported by Rahim and Keikhaei, **[25]** and Piplani et al., **[11]** who found significante increase of serum iron and serum ferritin among the studied cases with BTT (75.8 ±29.1 and 31.09 ±6.79 respectively) and (78.2 ± 18.3 and 36.1 ± 18.4 respectively) compared to the studied cases with IDA (20.03 ± 8.01 and 4.23±2.01 respectively) and (21.1 ±9.4 and 6.92±2.9 respectively). Taken in consideration that serum iron is non specific in the differentiation between patient with BTT and patient with IDA due to it is diurnal variation and patients with BTT have ineffective erythropoiesis due to imbalanced synthesis of globin chains and increase of total erythropoisis and plasma iron turnover leads to increased iron absorption, so it is considered as non significant parameter in differentiation between BTT and IDA.

Serum ferritin test is done for evaluating iron deficiency anemia as the ferritin levels measured usually have a direct correlation with the total amount of iron stored in the body. However, the result must be interpreted with caution in any patient with an underlying inflammatory process, as ferritin is an acute phase reactant, and is increased when an acute or chronic inflammatory process is present **[30]**.

In our studied cases TIBC which is a parameter related to iron deficiency was found significantly decreased among the cases with BTT (287.76 ± 71.62) compared to cases with IDA (497.55 ±60.29) with sensitivity 90.48% and specificity 100.00%. This in agreement with Rahim and Keikhaei, **[25]** and Demir et al., **[31]** who found that, significant decrease of TIBC level was found among cases with BTT (431.9±72.2 and 321.7±36.8 respectively) compared to cases with IDA (334.6 ±49.6 and 378.6 ±70.01) respectively. On the other hand Vehapoglu et al., **[20]** found, no significant differences between cases with BTT (339±40.47) compared to those with IDA (392±41.74).

Total iron-binding capacity measures the availability of iron binding sites. Extracellular iron is transported in the body bound to transferrin, a specific carrier protein. Hence, TIBC indirectly measures transferrin levels, which increase as serum iron concentration (and stored iron) decreases **[32]**.

Considering RBCs indices, our study showed significant decrease of RDW index among cases with BTT (156.08±22.10) compared to cases with IDA (273.85±71.16), with sensitivity 100%, specificity 79.3 1% AUC 0.897 and accuracy 88%. This is in agreement with Nesa et al., [21] who found that RDWI came out as good discriminator between BTT and IDA, with sensitivity 80.7% and specificity 84.7% in detection of BTT. And for detection of IDA were found 84.7% sensitivity and 80.7% specificity. These results are consistent with the findings of Demir et al., [31] and Sirdah et al., [33] who stated that, RDWI appears to be one of reliable and useful indices for initial screening of microcytic hypochromic anemia and is better than RDW% in differentiating IDA from BTT but is not the most reliable one as said by, Rahim and Keikhaei, [25] found that RDW index was one of the reliable indices in differentiation between BTT and IDA with sensitivity (97% and 71%) and specificity (71% and 97%) for BTT and IDA respectively but not the best one as Mentzer index was better than it with sensitivity (93% and 85% respectively) and specificity (85% and 93% respectively) for BTT and IDA, Piplani et al., [11] found that RDW index sensitivity (80.5% and 77.7% respectively) and specificity (77.7% and 80.5% respectively) for BTT and IDA respectively but was not the best one as Mentzer index was better than it with sensitivity (94.4% and 86.9% respectively) and specificity(86.9% and 94.4% respectively) for BTT and IDA respectively. Same result also had been found by Vehapoglu et al., [20] who found that, sensitivity of Mentzer index was (98.7% and 82.3% repectively) and specificity (82.3% and 98.7% respectively) for BTT and IDA and is better than RDWI with sensitivity (83.1% and 76.4% respectivly) and specificity (76.4% and 83.1% respectively) for differentiation between BTT and IDA respectively.

Our study showed significant decrease of Green and King index among the studied cases with BTT (49.63±8.45) compared to the studied cases with IDA (88.03±24.18), with sensitivity 95.24%, specificity 79.31%, AUC 0.873 and accuracy 86.00%. This is in agreement with the previous studies done by Ntaios et al., [24] who found that, Green and King proved to be a reliable index, as it had sensitivity 75.06%, specificity 95.8%, efficiency 80.12% for the detection of BTT, a similar result for the Green and King index was found by Urrechaga et al., [34] and Ferrara et al., [35] who found that, the Green and King index had the highest efficiency (80.2%) however, its validity is much lower compared to the original study on G & K by its authors, where the sensitivity of the index was 100% [13]. On the other hand Piplani et al., [11] found the sensitivity of this index was 79.2% and it showed a low specificity 69.9% ruling it out as a reliable indicator.

Our data showed significant decrease of Mentzer index among cases with BTT (11.73 ± 1.27) compared to cases with IDA (14.85 ±2.02), with sensitivity 95.24%, specificity 93.1%, AUC 0.942 and accuracy 94.00%, this with is in agreement with Piplani et al., [11] who found that, Mentzer index showed a reasonably high sensitivity 94.4% and specificity 86.9% in differentiation between BTT and IDA. Ehsani et al., [36] stated that, the best discrimination index for differentiation between BTT and IDA is Mentzer index with sensitivity 95.5% and specificity 94.6% for BTT, and sensitivity 94.6% and specificity 95.5% for IDA. Vehapoglu et al., [20] also found that Mentzer index had the high sensitivity 98.7%, specificity 82.3% for correctly distinguishing BTT and IDA. When the Mentzer index was calculated, 91% of children with microcytic anemia were correctly diagnosed. Rahim and Keikhaei, [25] also found that, Mentzer index had 85% sensitivity and 93% specificity. Similar results of Mentzer index sensitivity 90.9% and specificity 80.3% were found by Ghafouri et al., [37] . In contrast Demir et al., [31] found that, sensitivity of Mentzer index (62% and 86%) and it is specificity (86% and 62%) for BTT and IDA respectively, and according to their results Mentzer index cannot be considered as one of the reliable indices and RDW index followed by RBCs count are better than it. Also, Sirdah et al., [33] found that the most reliable indices for differentiation between BTT and IDA are Green and King index followed by RDW index. According to Ntaios et al., [24] Green and King shows the highest reliability, followed by England and Fraser, RBC count, Mentzer index, and RDW index.

Conclusion:

Among the studied indices, reduced Mentzer index <13 showed the highest diagnostic performance for discrimination between iron deficiency anemia and beta thalassemia trait by detection of increased HbA_2 >3.5% as proved by ROC test. While among the studied parameters, TIBC ≤350 µg/dl showed the highest diagnostic performance for this discrimination, as proved also by ROC test.

Financial support and sponsorship:

Nil.

Recommendations:

As discrimination between iron deficiency anemia and beta thalassemia trait is very essential to reach the suitable therapeutic strategy for such cases, therefore, we recommend the use of Mentzer index as a feasible, costless method with a high diagnostic performance for preliminary discrimination between this two diseases.

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الآداء التشخيصى لنطاق توزيع الخلايا الحمراء ومؤشر منتزر (Mentzer) للتمييز بين آنيميا نقص الحديد وسمة الثلاثيميا بيتا

تعتبر آنيميا كريات الدم الصغيرة الشاحبة هى آشهر آنواع فقر الدم المنتشرة فى آنحاء العالم وتندرج تحت هذا النوع من الآنيميا آنواع آخرى إما آن تكون وراثية مثل آنيميا البحر المتوسط وبعض آمراض الهيموجلوبين آو مكتسبة كآنيميا نقص الحديد.

وتكمن آهمية الحديد فى آنه يعتبر من آهم المعادن التى تدخل فى تكوين جسم الإنسان، وبالتالى نقص الحديد يعتبر من آهم وآشهر آمراض سوء التغذية فى الإنسان حيث آنه يصيب حوالى ١.٦ بليون شخص على مستوى العالم ومعظمهم يعيشون فى البلدان النامية، قد تؤدى آنيميا نقص الحديد آيضا إلى ضعف القدرة على التحمل البدنى، والقدرة على العمل، وتطور نمو الجنين، وقدره الجهاز المناعى. ويؤدى إنخفاض مخازن الحديد فى المخ إلى خلل نشاط الإنزيمات التى تعتمد عليه وتدهور الناقلات العصبية لها.

آما آنيميا البحر الآبيض المتوسط فهى آحد آمراض فقر الدم المزمنة، التى تؤثر على صنع كريات الدم الحمراء، فتكون مادة الهيموجلوبين غير قادرة على القيام بمهمتها فى نقل الآكسجين للجسم، بالإضافة إلى رفع مستوى الحديد فى الدم، ويسمى المرض آيضا (الثلاثيميا). والمصاب بالثلاثيميا الصغرى آو سمة الثلاثيميا بيتا فى الغالب لا يعانى من آى آعراض آو تكون الآعراض الخفيفة جدا مثل فقر دم خفيف، ويتم تشخيص الحالة بإجراء فحص روتينى للدم يظهر إنخفاضا فى كريات الدم الحمراء. وتكمن آهمية تشخيص المرض للمساعدة فى تحديد خطورة إنتقال الثلاثيميا الكبرى للآطفال عند الزواج. وتعتبر سمة الثلاثيميا بيتا المسبب الثانى لآنيميا كريات الدم الصغيرة بعد آنيميا نقص الحديد. ونظرا للتشابه الكبير بين آنيميا نقص الحديد وسمة الثلاثيميا بيتا من الناحية المخبرية قد يؤدى إلى التشخيص الخاطئ.

تقييم الآداء التشخيصى لآساليب بسيطة وغير مكلفة وهى عرض تورزيع الخلايا الحمراء (RDWI)، ومؤشر عرض توزيع الخلايا الحمراء (RDW%)، ومؤشر منتزر (Mentzer) للتمييز بين آنيميا نقص الحديد وسمة الثلاثيميا بيتا، من العيادات الخارجية عيادة آمراض الدم بمستشفى الآطفال جامعة آسيوط خلال فترة من ١ يونيو ٢٠١٦ حتى نهاية شهر مايو ٢٠١٧.

آظهر مؤشر منتزر (Mentzer) من بين المؤشرات المدروسة، آعلى آداء تشخيصى فى التمييز بين آنيميا نقص الحديد وسمة الثلاثيميا بيتا عن طريق الكشف عن زيادة $Hb\ A_2 > ٣.٥٪$ كما آثبته إختبار (ROC). فى حين آنه من بين كل العوامل المدروسه، آظهر عامل TIBC ≥٣٥٠٪ ميكروجرام/ديسيليتر آعلى آداء تشخيصى فى التمييز بين آنيميا نقص الحديد وسمة الثلاثيميا بيتا كما آثبته آيضا إختبار ROC.