http:// ijp.mums.ac.ir
Original Article (Pages: 15285-15295)

Hematological indices in differentiation between iron deficiency anemia and beta-thalassemia trait

Nagwan I. Rashwan[1], Ahmed El-Abd Ahmed[1], *Mohammed H. Hassan[2], Maha E. Mohammed[1], Ali Helmi Bakri[1]

[1] Pediatric Department, Faculty of Medicine, South Valley University, Qena83523, Egypt.

[2] Medical Biochemistry Department, Faculty of Medicine, South Valley University, Qena83523, Egypt.

Abstract

Background: Mild microcytic hypochromic anemias caused by iron deficiency (IDA) and beta-thalassemia trait (β-TT) continue to be problematic and a significant threat to society, particularly in relatively poor developing countries. The goal of this study was to use an accuracy approach to improve the diagnostic function of eight different prejudiced indices in Egyptian pediatric patients with microcytic anaemia.

Methods: Eight discrimination indices for diagnostic performance are introduced to analyze the differences between β-β-TTand IDA among Egyptian paediatric patients by using evaluation metrics calculated from RBC indices by various mathematical formulae. CBC, iron study and hemoglobin electrophoresis were performed to all included participants.

Results: A total of 300 Egyptian paediatric patients with β-TT or IDA were enrolled. The Mentzer and Ehsani index exhibited the highest diagnostic accuracy (100%) followed by Sirdah (97.5%), Sirvistava (95%), MDHL (92.5%) Green & King, Recierca, (90%), and Matos (70%). Indices with AUCs greater than 0.8, such as Mentzer, Ehsani, and Sirdah had very valuable predictive accuracy in distinguishing between β-TT and IDA.

Conclusion: Although Hb electrophoresis is the gold standard for diagnosing β-TT, in developing countries, the Mentzer index, followed by the Ehsani and Sirdah indices, could be used as a simple and cheap method to distinguish β-TT from IDA in pediatric patients with mild microcytic hypochromic anaemia.

Key Words: Beta-thalassemia trait, Egyptian children, Hematological indices, Iron deficiency anemia.

* Please cite this article as: Rashwan N, El-Abd Ahmed A, Hassan M, Mohammed M, Helmi Bakri A. Hematological indices in differentiation between iron deficiency anemia and beta-thalassemia trait. Int J Pediatr 2022; 10 (1): 15285-15295. DOI: **10.22038/IJP.2021.59644.4644**

**** Corresponding Author:***

Mohammed H. Hassan, Associate Professor of Medical Biochemistry, Faculty of Medicine, South Valley University, Qena 83523, Egypt. Email: Mohammedhosnyhassaan@yahoo.com

Received date: Aug.12,2021; Accepted date:Oct.30,2021

1. INTRODUCTION

The most frequent causes of hypochromia and microcytosis anaemia in pediatrics are Iron Deficiency (ID) and βeta-Thalassemia Trait (β-TT). ID is the high incidence anaemia which is placed among the top 20 risks for the global distribution of disease burden; and is the world's most common dietary disorder. In Egypt, Iron Deficiency Anaemia (IDA) is a significant public health issue (1).

The most common type of hereditary hemoglobinopathy is βTT. It is estimated that approximately half of the world's population have βTT. Although it is asymptomatic and usually not linked with any clinical handicap, it is critical to detect it early in order to avoid the birth of a child with homozygous thalassemia syndromes (2).

It's tough to tell the difference between IDA and β-TT because their morphological findings are so similar. The distinction between IDA and β-TT is critical for two reasons. First, if β-TT is misdiagnosed as IDA and unwanted iron is prescribed by the attending physician, Hb will not improve. The second compelling problem is that a β-TT misconceived as IDA may marry a β-TT, resulting in homozygous or thalassemia major offspring (3).

To distinguish between IDA and β-TT patients, numerous indices and equations including red cell indices derived from automated red cell analyzers, such as RBC, haemoglobin (Hb), mean cell volume (MCV), and red cell distribution width (RDW) in specific materials have been published. In the distinction of IDA and β-TT; however, none of these indices possess a sensitivity (SENS) and specificity (SPEC) of 100 %. Furthermore, there are only a few studies assessing the reliability of different methods in the pediatric age group (4). The gold standard for diagnosing IDA and β-TT is serum iron, serum ferritin level, and HBA2 estimation. However, these metrics are costly and time-consuming for the public health financial system, particularly in global prevalence of microcytosis and hypochromia. In addition, except for automated blood cell counts, physicians in our country do not have the capability to enhance these metrics. The purpose of this study is to ascertain the clinical utility and validity of some RBC indices and formulas in the delineation of β-TT and IDA in a pediatric population of Qena Governorate patients; and to determine which indicators are the best for reducing unnecessary inquiry costs put on the healthcare system and are more affordable, as well as less costly and complex.

2- MATERIALS AND METHODS

2-1. Study design and participants

This cross sectional study included 300 patients (130 males and 170 females) ,who were diagnosed with microcytic hypochromic anemia by CBC at Pediatric outpatient clinics, Qena University hospitals, SouthValley University, Egypt, between January 2018 and December 2018. The Ethical Committee affiliated with the Qena Faculty of Medicine, South Valley University, authorised and approved this study, which was carried out in accordance with the principles of the Helsinki Declaration. Before the initiation of the study, informed consents were obtained from the guardians of the participating children.

2-2. Patients' selection criteria

The inclusion criteria are based on the definition of anemia as Hb levels between 8.7–11.4 gram/deciliter, microcytosis defined as MCV 80 FL in children above the age of 6 years or MCV 70 FL in children under the age of 6 years, and hypochromia defined as an MCH level of 30 g/dL at age 2 and 31 g/dL at any other age. Hence the following cases were excluded from the study: The Children

refracting to iron supplementation, receiving iron replacement for three months or more without clinical or laboratory improvement, who had stopped iron therapy for one month at least**; t**he children with acute or chronic inflammation, infectious disease, acute bleeding or receive a blood transfusion in the previous three months; and the children with lead poisoning or anemia with hemoglobin less than 7 gm/dl, all.

2-3. Laboratory workup

After a 30-minute rest in a sitting position, the children's fasting venous blood samples were collected. Each child had two blood samples taken. One of the samples was gathered in a Vacutainer EDTA-K2 tube and used for a complete blood count (CBC) test and HbA2 analysis. The serum was extracted from the other sample, centrifuged for 5 minutes. Using this serum, serum iron, serum ferritin, and total iron-binding capacity were all determined.

2-3-1. Complete blood count

The CBC test was performed using a cell dyne-Ruby (Abbott Diagnostics-Santa Clara-California-USA-Abbott 2010) automated cell counter equipped with the necessary tester, resins, and verification.The discrimination variables were computed, validated, and correlated to distinguish between β-TT and IDA **(Table 1)**, (5-13).

Table-1: Indicators of discriminatory practices for the distinction between β-TT and IDA in microcytic anaemia patients

Indices	Calculation	Cut–off β-TT	Cut–off IDA	Reference
Mentzer	MCV/RBC	<13	>13	(5)
Srivastava	MCH/RBC	<3.8	>3.8	(6)
Ricerca	RDW/RBC	<4.4	>4.4	(7)
Green and King	$(MCV^2 \times RDW)/(100\ HB)$	<65	>65	(8)
MDHL (Mean Density of Hb/liter of Blood)	(MCH/MCV) × RBC	>1.63	<1.63	(9)
Matos& Carvehllo (MCI)	(1.91 × RBC) + (0.44 × MCHC)	>23.85	<23.85	(10)
Sirdah	MCV − (3 × Hb)	<27	>27	(11)
Ehsani	MCV − (10 × RBC)	<15	>15	(12)

2-3-2. Iron study and electrophoresis:

A) In terms of two standard tests, Iron study profile and Hb electrophoresis, all patients were categorized either as IDA or β-TT. The assays of serum iron and total iron binding capacity, using T60 UV visible spectrophotometer. PG INSTRUMENTS LIMITED, Alma park wibtoft, Leicestershireshire, England. LE17SBE, Serial No. 20-1650-01-0010, were performed using commercially available colorimetric assay kit supplied by Spectrum Diagnostics Co. Cairo, Egypt, Catalog No: 270001. Serum ferritin was measured by microplate ELISA reader (EMR-500, Labomed. Inc., USA) using commercially available ELISA assay kit (Bio Check USA catalog number BC-1025).

B) Hemoglobin electrophoresis was performed by (Bio-Rad dual D 10 dual program record pack, Ref 220-0201 CA 94547) with the necessary calibrator, resins, and quality control procedures.

2-4. Statistical analysis

SPSS version 19 was used for data entry and analysis. The information was

presented in the form number, percentage, mean, median, and standard deviation. In order to compare qualitative variables, the Chi-square and Fisher exact tests were used; and to compare the two quantitative variables, Mann-Whitney u test was utilised. To compute sensitivity, specificity, along with the positive and negative predictive values, the Med calc program was utilised. P-value<0.05 was regarded as statistically significant.

3- RESULTS

3-1. Demographic data of the included participants

The study included 300 children of both sexes (130 males, 45% of whom had HBA2 levels greater than 3.5%, and 40% of whom had HBA2 levels less than 3.5% were included in the IDA group) and (170 females, 55% of whom had HBA2 levels greater than 3.5% were included in the IDA group; and 60% of them who had HBA2 levels less than 3.5% were included in the IDA group).

3-2. Haematological parameters and iron profile of the study groups

The RBC count was discovered to be greater in patients of β-TT (4.22 - 5.79 $x10^6$/ µlwith the mean of $5.37x10^6$/ µl±0.43) than in IDA patients (3.1-$4.68x10^6$/ µl). Hb levels fell in the β-TT group to 9.64±0.61g/dl and in the IDA group to 8.87±0.74g/dl (P=0.005) (**Table 2**). MCV, MCH, and MCHC variations between β-TT and IDA were determined to be considerable (p=0.001). The mean values for serum iron and serum ferritin were significantly lower in the IDA group compared to the β-TTgroup (P=0.031 and P=0.001, respectively), while TIBC showed an opposite trend (P=0.001) (**Table 2**).

3-3. Values of the studied hematological indices among the included participants

The data showed a significant decrease of Mentzer, Ehsani, Sirdah, Green and King, Ricerca , and Sirvistava indices in the β-TT group (10.7±1.13, 3.47±5.51, 22.85±4.4, 62.48±18.8, 3.39±0.77, 196.44±53.78, and 3.46±0.67 respectively) with P<0.001 compared to IDA group, while there was a significant increase in MDHL index in the β-TT group (1.72±0.2) with P<0.001 compared to IDA group (**Table 3**).

Table-2: Hematological and Biochemical Parameters of study groups

The studied parameters	Beta- thalassemia trait (n=200)		Iron Deficiency Anemia (n=100)		P. value
	Mean ± SD	Range	Mean ± SD	Range	
Age (yrs)	6.1±3.23	3 - 10.5	4.38±2.26	1 - 8	0.144
RBCs (X106/ µl)	5.37±0.43	4.22 - 5.79	3.89±0.53	3.1 - 4.68	<0.001**
HB (g/ dl)	9.64±0.61	7.4 - 10.6	8.87±0.74	7.7 - 9.7	0.005**
MCV (fL)	57.13±3.87	45.8 - 63	65.32±2.74	61.3 - 69.5	<0.001**
MCH (pg)	18.38±1.86	15 - 25.1	21.86±1.89	18.9 - 25.9	<0.001**
MCHC(g/dl)	30.97±1.28	27.4 - 33.1	32.1±4.98	21 - 40	<0.001**
RDW (%)	18.06±3.51	11.6 - 25	18.76±2.77	16.5 -25.8	0.584
SI (µg/dl)	73.35±39.53	42 - 170	38.85±38.81	7 - 113	0.031*
SF (ng/ml)	37.22±24.03	10.52 - 104	7.39±4.67	3 - 18.4	0.001**
TIBC(µg/dl)	305.7±35.97	250 - 410	476.1±43.49	400 - 530	<0.001**

List of abbreviations: (RBCs=Red Blood Cell Count, Hb= Haemoglobin, MCV =Mean corpuscular volume, MCH= Mean corpuscular haemoglobin, MCHC= Mean corpuscular haemoglobin concentration, RDW=Red cell distribution width, SI= serum iron, SF= serum ferritin and TIBC= total iron binding capacity).

Table-3: Discriminating indices values of study groups

Discriminant indices	Beta-thalassemia trait (n=200)		Iron Deficiency Anemia (n=100)		P. value
	Mean ± SD	Range	Mean ± SD	Range	
Mentzer	10.7±1.13	9.5 - 12.7	17.11±2.75	13.2 - 21.74	<0.001**
Ehsani	3.47±5.51	-2.5 - 13.5	26.46±6.72	14.5 - 37.5	<0.001**
Sirdah	22.85±4.4	9.78 - 29.1	34.82±3.33	28.4 - 40.6	<0.001**
Matos & Carvaiho	33.58±45.56	21.4 - 227.1	21.53±2.48	16.3- 24.74	0.414
MDHL	1.72±0.2	1.34 - 2.31	1.3±0.2	0.93 - 1.53	<0.001**
Green and king	62.48±18.8	27.9 - 100.27	90.82±16.22	70.7 - 123.64	<0.001**
Ricerca	3.39±0.77	2.34 - 4.87	4.9±0.96	3.8 - 6.29	<0.001**
Srivastava	3.46±0.67	2.9 - 5.94	5.7±0.97	4.2 - 6.93	<0.001**

3-4. Performance of discriminatory indices for differentiating β-TT from IDA in the study groups

The diagnostic accuracy of discriminatory indices for differentiating β-TT from IDA in the study groups with microcytic anaemia for tracking the increased HbA2 (>3.5 percent) were listed based on the sensitivity (TPR), specificity (TNR), positive and negative predictive values (PPV and NPV), and accuracy, 95% confidence interval, and area under the curve (AUC). Our data show that Mentzer, Ehsani, Matos index presents the highest sensitivity (100%) followed by Sirdah and MDHL (95%), Sirvistava (90%), Green & King, and Recierca (80%) (**Table 4**).

The highest PPV is obtained with Mentzer, Ehsani, Sirdah, Green & King, Recierca, and Sirvistava (100%) followed by MDHL (95%) and Matos (76.9%). The lowest NPV is obtained with Green & King, and Recierca (71.4%) followed by Sirvistava (83.3%), MDHL (90%), Sirdah index (90.9%), Mentzer, Ehsani and Matos (100%) (**Table 4**).

Table-4: The diagnostic effectiveness of the investigated indices for detecting elevated HbA2 in patients with iron deficiency anaemia and beta-thalassemia trait

	Optimal cut off value	Sensitivity	95% CI of Sensitivity	Specificity	95% CI of Specificity	PPV %	NPV %	AUC	95% CI of AUC	Accuracy %
Mentzer index	≤12.7	100.0	83.2-100.0	100.0	69.2-100.0	100.0	100.0	1.000	0.884-1000	100.0
Ehsani index	≤13.5	100.0	83.2-100.0	100.0	69.2-100.0	100.0	100.0	1.000	0.884-1.000	100.0
Sirdah index	≤28.17	95.0	75.1-99.9	100.0	69.2-100.0	100.0	90.9	0.995	0.875-1.000	97.5
Matos and Carvehllo index	>21.29	100.0	83.2-100.0	40.0	12.2-73.8	76.9	100.0	0.675	0.480-0.834	70.0
MDHL index	>1.49	95.0	75.1-99.9	90.0	55.5-99.7	95.0	90.0	0.970	0.833-0.999	92.5
Green and king index	≤67.3	80.0	56.3-94.3	100.0	69.2-100.0	100.0	71.4	0.885	0.716-0.972	90.0
Ricerca index	≤3.36	80.0	56.3-94.3	100.0	69.2-100.0	100.0	71.4	0.880	0.709-0.969	90.0
Srivastava index	≤3.92	90.0	68.3-98.8	100.0	69.2-100.0	100.0	83.3	0.968	0.829-0.999	95.0

List of abbreviation: AUC= area under curve, ACC= accuracy, PPV = positive predictive value, NPV = negative predictive value, 95% CI= 95% confidence interval

Furthermore, Mentzer, Ehsani, Sirdah, Green & King, Recierca and Sirvistava show the highest specificity (100%), followed by MDHL (90%) and Matos (40%).

Concerning the diagnostic accuracy (ACC) for detection of increased HbA2 (>3.5%): Mentzer and Ehsani were of the highest ACC (100%) followed by Sirdah (97.5%), Sirvistava (95%), MDHL (92.5%) Green & King, Recierca, (90%), and Matos (70%). In addition, Mentzer, and Ehsani showed the highest AUC with 95% CI (0.884 – 1000) followed by Sirdah 0.995 with 95% CI (0.875 - 1.000), MDHL (0.970) with 95% CI (0.833 - 0.999), Sirvistava (0.968) with 95% CI (0.829 - 0.999), Green and King (0.885) with 95% CI (0.716 - 0.972), Recierca (0.880) and Matos (0.675) with 95% CI (0.709 - 0.969/ 0.480 - 0.834, respectively) (**Table 4**).

A Receiver Operator Curve (ROC) was created to assess the diagnostic efficacy of the investigated indices in detecting elevated Hb A2 (> 3.5 percent) in the IDA and β-TT groups. (**Fig. 1 and 2**). The optimal cut-off value of the studied indices were found to be as follows: Mentzer, Ehsani, Sirdah, Matos, MDHL, Green & king, Recierca, and Srivastava Were (≤12.7, ≤13.5, ≤28.17, >21.29, >1.49, ≤67.3, ≤3.36, and ≤3.92, respectively) (**Fig. 1 and 2**).

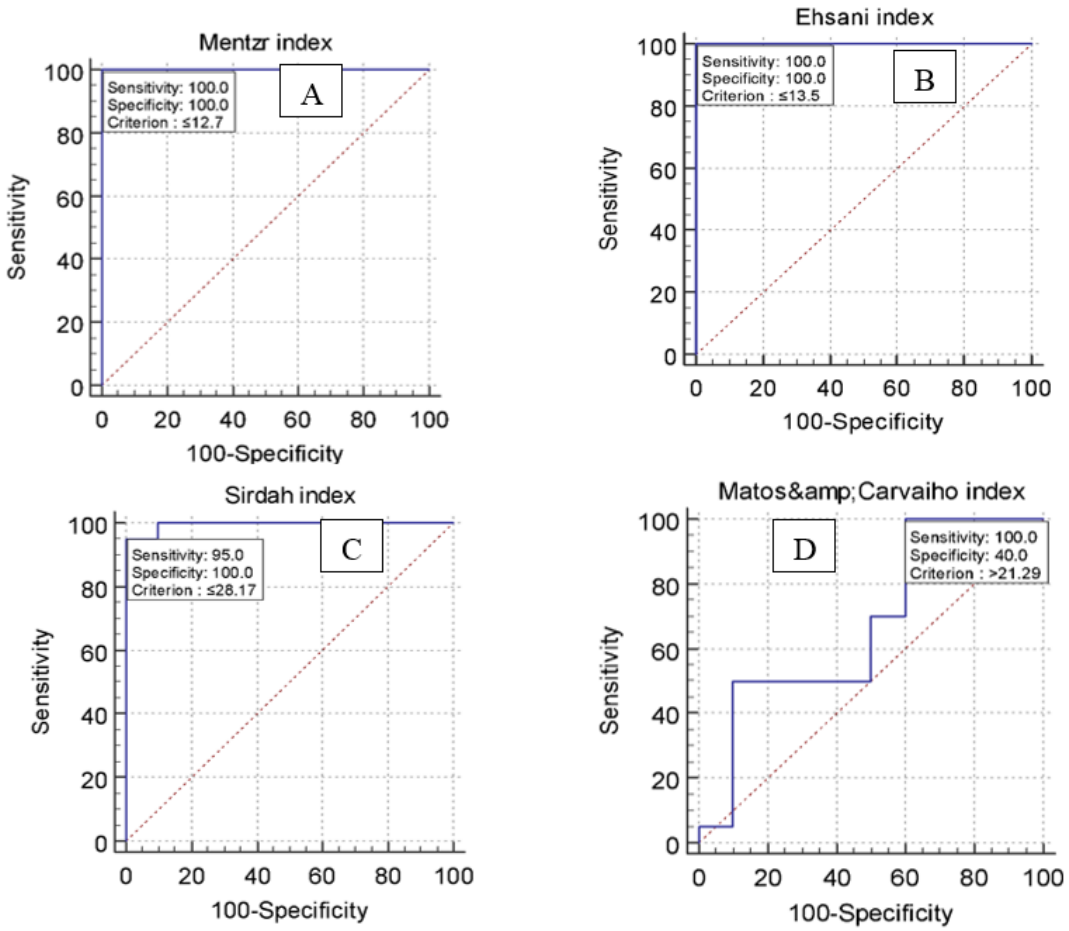

Fig. 1: (A), Receive operator characteristics (ROC) curve for determining Mentzer's clinical utility. **(B)**, Receiver operator (ROC) curve to assess Ehsani's clinical utility. **(C)**, Receiver operator (ROC) curve to assess Sirdah's clinical utility. **(D)**, Receiver operating characteristic (ROC) curve to assess Matos' diagnostic ability for detecting elevated Hb A2 blood levels (3.5 percent) between the investigated groups with βTT and IDA

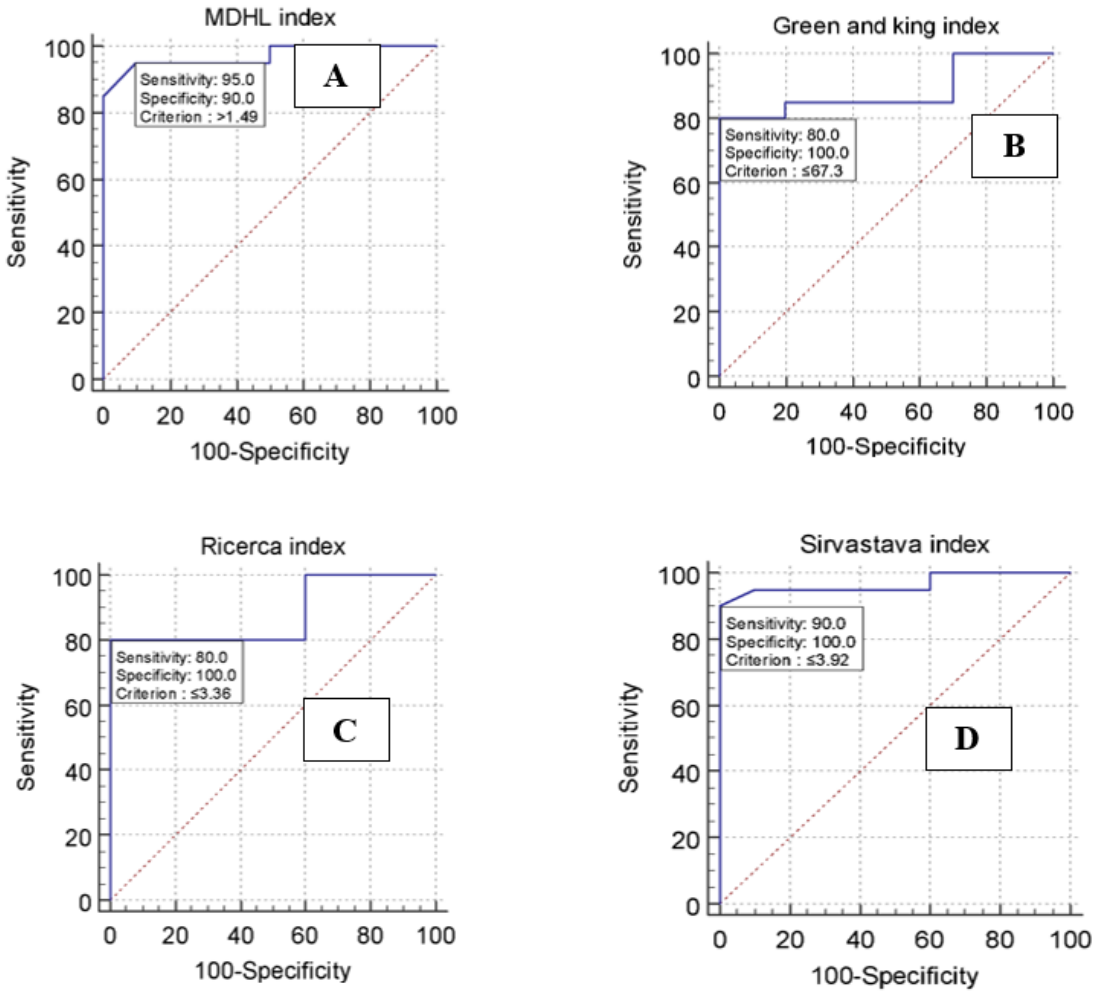

Fig. 2: (A), Receiver operator characteristics (ROC) curve for determining MDHL classification accuracy. **(B)**, Receiver operating characteristic (ROC) curve to assess Green& King's classification accuracy. **(C)**, Receiver operating characteristic (ROC) curve to assess Recierca's classification accuracy. **(D)**, Receiver operating characteristic (ROC) curve to assess Sirvistava's classification accuracy. For the identification of higher Hb A2 blood levels (3.5%) between the investigated groups with βTT and IDA

4- DISCUSSION

β-TT and IDA are two prevalent causes of microcytic anaemia recognised by pediatrics. These two hematologic diseases are comparable in different clinical and research aspects. Misdiagnosis of β-TT has ramifications for possible homozygous progeny. Additionally, in young children, IDA with or without explicit anaemia can cause substantial delays in cognitive function, which may be resolved by early iron treatment.

Until date, the appropriate screening approach for β-TT has been based on an increase in HbA2, while the diagnosis of IDA has been based on a rise in TIBC, a reduction in blood iron, and a serum ferritin rise. Due to the significance of distinguishing between β-TT and IDA, several different indices were suggested in the market, using only a complete blood count. These indices demonstrated varying clinical utilities, but none of them had a confirmed diagnosis in various studies;

and especially no study has confirmed the validity of any of these RBC indices (14).

We proposed eight haematological indices discriminating between IDA and β-TT, using CBC and iron profile characteristics, which provided us with strong clinical significant, accurate, quick, inexpensive, and easy tests to identify children at high risk of these anemias and avoid needless investigative expenditures. In this situation, a dependable discrimination index becomes critical.

It is feasible to distinguish between β-TT and IDA without employing expensive procedures with high-performance indices; our data revealed substantial differences in the haematological parameters of the β-TT and IDA categories, with the exception of RDW, which agrees with Vehapoglu et al. (2).

The studied parameters showed a significant decrease in hemoglobin concentration among the studied cases between IDA and βTT, but with higher values of βTT in comparison to IDA that showed agreement with Beyan et al. (15) and Rathod et al. (16).

RBC count has been regarded a valuable measure; in our study, RBC count revealed a substantial rise in the β-TT group compared to the IDA group, which is consistent with Demir et al.(17) and Sanjay Piplani et al.(18); However, RBC count alone is deemed to be an unreliable indicator for distinguishing between β-TT and IDA.

The current study demonstrated a substantial drop in MCV, MCH, and MCHC among the investigated patients in β-TT compared to IDA, which is comparable to Beyan et al. (15), and Yousafzai et al. (19), who discovered that MCV and MCH were lower in the β-TT group than those observed in the IDA group. As a result, MCV and MCH values were discovered to be useful factors for identifying β-TT. This finding is in contrast to that of Jameel et al.(20) , who reported that MCV, MCH, and MCHC values did not differ significantly across groups.

According to iron study, our data showed a significant decrease of serum iron and ferritin between cases of IDA compared to β-TT while a significant increase of TIBC value was found among the IDA cohort matched to the β-TT group, that is in agreement with Jameel et al. (20) and Pornprasert et al. (21).

The ideal index for beta-thalassemia trait should have a very high sensitivity as well as a relatively good specificity, allowing it to detect the greatest number of individuals with β-TT while excluding those with IDA (high sensitivity) (high specificity). In line with the results of many studies (22-25,2,12,18), the current study indicated that the Mentzer and Ehsani indices were substantially higher, more dependable, and more accurate in differentiating between β-TT and IDA with high sensitivity, specificity, and AUC (100% sensitivity,100% specificity, and 1.00 AUC) for both. However, Demir et al (17) proved that Mentzer index had low validity in pediatric patients, which is against our findings.

Similar to Vahapogla et al. (2) and Telmissani et al. (9), our results demonstrated that Sirdah, Sirvistava, and MDHL indices were significant and valid in differentiation between IDA and βTT with respectively, 95%, 90%, and 95% sensitivities; 100%, 100%, and 90% specificities; and 0.995, 0.968 and 0.970 AUCs. However, in contrast to our findings, Keikhaei et al. found MDHL to be an invalid index (25).

Urrechage et al. (24), In contrast to Vehapoglu et al. (2), found that Recierca index is a reliable index for differentiating between IDA and βTT.The Ricerca index has a sensitivity of 100 percent but a

specificity of just 16.9 percent, ruling it out as a trustworthy predictor.

According to our study, the Green and King index were significant with the sensitivity of 80%, specificity of 100%, and AUC of 0.885. Likewise, Urrechaga et al.(24), Ferrara et al. (26), and Sirdah et al. (11) found that Green and King is an accurate discriminating index.

The present study proved that Matos and Carvehllo Index (MCI) was invalid in discrimination between βTT and IDA with 100% sensitivity and specificity; and with an AUC of 0.675 that was in agreement with Urrechage et al (24). However, Matos et al. (10) discovered that MCI had great agreement with the gold standard techniques of diagnosis for these anemias.

Roc curves are useful for comparing the diagnostic effectiveness of various tests. The AUC value is unaffected by the cut-off values, making it more trustworthy. Our study proved, consistent with the value of the AUC, that Mentzer, and Ehsani indices showed the highest AUC with 95% CI (0.884 – 1000) compatible with Ghafouri et al. (22) and Ehsani et al. (12).

However, research studies in paediatric age ranges are rare, and their findings are contradictory. We discovered inconsistent results with some other prior publications, which might be due to the varied molecular spectrums of -thalassemia disease in different nations, or even in different areas of the same country. It might be explained by the differences in sample sizes. Another explanation might be related to the omission of IDA individuals with lower Hb levels from the analyses, in some studies.

5- CONCLUSION

This cross-sectional study was conducted on pediatric patients referring to Pediatrics' Clinics of Qena University Hospitals, Egypt, with microcytic anemia. Based on the findings, it can be concluded that CBC based indices (particularly Mentzer, Ehsani,Green and King, MDHL, Recierca, Sirdah, and Sirvistava indices) were reliable with valid diagnostic performance in differentiating β-TT from IDA in developing countries with limited health-care resources.

6- STUDY LIMITATION

Limitations of the current study include the limited sample size and the absence of analyses of all red cell indices. There is a need for more research, testing the distinguishing function of all potential red cell indices previously reported so far; however, the current study allows us to employ seven of them.

7- FUNDING

The current research was funded by the authors themselves.

8- COMPETING INTERESTS

None.

9- REFERENCES

1. Mousa SMO, Saleh SM, Higazi AMM, Ali HAA. Iron Deficiency and Iron Deficiency Anemia in Adolescent Girls in Rural Upper Egypt.International Blood Research & Reviews 2016; 5:1-6. 10.9734/IBRR/2016/25826.

2. Vehapoglu A, Ozgurhan G, Demir AD, Uzuner S, Nursoy MA, Turkmen S, Kacan A. Hematological indices for differential diagnosis of Beta thalassemia trait and iron deficiency anemia. Anemia. 2014; 2014:576738.

3. Jameel T, Baig M, Ahmed I, Hussain MB, Alkhamaly MD. Differentiation of beta thalassemia trait from iron deficiency anemia by hematological indices. Pak J Med Sci. 2017; 33(3):665-669. DOI: https://doi.org/10.12669/pjms.333.12098.

4. Nalbantoğlu B, Güzel S, Büyükyalçın V, Donma MM, Güzel EÇ, Nalbantoğlu A, Karasu E, Özdilek B. Indices used in

differentiation of thalassemia trait from iron deficiency anemia in pediatric population: are they reliable? Pediatr Hematol Oncol. 2012 Aug; 29(5):472-8. doi: 10.3109/08880018.2012.705230. PMID: 22866672.

5. Mentzer WC Jr. Differentiation of iron deficiency from thalassaemia trait. Lancet. 1973 Apr 21; 1(7808):882. doi: 10.1016/s0140-6736(73)91446-3. PMID: 4123424.

6. Srivastava PC, Bevington JM. Iron deficiency and-or thalassaemia trait. Lancet. 1973 Apr 14; 1(7807):832. doi: 10.1016/s0140-6736(73)90637-5. PMID: 4121255.

7. Ricerca BM, Storti S, d'Onofrio G, Mancini S, Vittori M, Campisi S, Mango G, Bizzi B. Differentiation of iron deficiency from thalassaemia trait: a new approach. Haematologica. 1987 Sep-Oct; 72(5):409-13. PMID: 3121463.

8. Green R, King R. A new red cell discriminant incorporating volume dispersion for differentiating iron deficiency anemia from thalassemia minor. Blood Cells. 1989; 15(3):481-91; discussion 492-5. PMID: 2620095.

9. Telmissani OA, Khalil S, George T R. Mean density of hemoglobin per liter of blood: a new hematologic parameter with an inherent discriminant function. Laboratory Haematology. 1999; 5:149–152.

10. Matos JF, Dusse LM, Borges KB, de Castro RL, Coura-Vital W, Carvalho Md. A new index to discriminate between iron deficiency anemia and thalassemia trait. Rev Bras Hematol Hemoter. 2016 Jul-Sep; 38(3):214-9.

11. 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-thalassaemia minor from iron deficiency in Palestinian population. Int J Lab Hematol. 2008 Aug; 30(4):324-330.

12. Ehsani M, Shahgholi E, Rahiminejad, M, Seighali F, Rashidi A. A new index for discrimination between iron defciency anemia and beta-thalassemia minor: results in 284 patients. Pakistan journal of biological sciences: PJBS 2009; 12: 473–475.

13. Mohammed ME, Ahmed A E, Hassan MH, Rashwan NI. RDW for Discrimination between Iron Deficiency Anemia and Beta Thalassemia Trait among cohort of Egyptian children in Upper Egypt. SVU-International Journal of Medical Sciences. 2019; 2(2): 64-68.

14. Jahangiri M, Rahim F, Malehi A S. Diagnostic performance of hematological discrimination indices to discriminate between βeta thalassemia trait and iron deficiency anemia and using cluster analysis: Introducing two new indices tested in Iranian population. Sci Rep 9, 18610 (2019). https://doi.org/10.1038/s41598-019-54575-3.

15. Beyan C, Kaptan K, Ifran A. Predicive value of discrimination indices in differential diagnosis of iron deficiency anemia and beta thalassemia trait. EurJ Hematol2007; 12(1):81-84.

16. Rathod DA, Kaur A, Patel V,Patel K,Kabrawala R,Patel V et al. Usefulness of cell counter based parameters and formulas in detection of beta thalassemia trait in areas of high prevelance.Am J Clin Pathol 2007; 128:585-589.

17. 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):612-6.

18. Piplani1 S, Manan R, Lalit M, Manjari M, Bhasin T, Bawa J. NESTROFT - A Valuable, Cost Effective Screening Test for Beta Thalassemia Trait in North Indian

Punjabi Population. JCDR. 2013; 7(12):2784-2787.

19. Yousafzai YM, Khan S, Raziq F.Beta thalassemia trait: hematological parameters. 2010; 22(4):84-6.

20. Jameel T, Baig M, Ahmed I, Hussain MB, Alkhamaly MBD. Differentiation of beta thalassemia trait from iron deficiency anemia by hematological indices. Pak J Med Sci. 2017 May-June; 33(3):665-669.

21. Pornprasert S, Panya A, Punyamung M, Yanola J, Kongpan C. Red cell indices and formulas used in differentiation of β-thalassemia trait from iron deficiency in Thai school children. Hemoglobin. 2014; 38(4):258-61.

22. Ghafouri M, Mostaan S L, Sharif S, Hosseini G L, Atar C Z. Comparison of cell counter indices in differentiation of beta thalassemia minor from iron deficiency anemia. (2006).

23. Jameel T, Baig M, Ahmed I, Hussain MB, Alkhamaly MBD. Differentiation of beta thalassemia trait from iron deficiency anemia by hematological indices. Pak J Med Sci. 2017; 33(3):665-669.

24. Urrechaga E, Borque L, Escanero JF. The role of automated measurement of RBC subpopulations in differential diagnosis of microcytic anemia and β-thalassemia screening. Am J Clin Pathol. 2011 Mar; 135(3):374-9.

25. Rahim F, Keikhaei B. Better differential diagnosis of iron deficiency anemia from beta-thalassemia trait. Turkish Journal of Hematology. 2009; 26(3):138–145.

26. Ferrara M, Capozzi L, Russo R, et al. 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.