Original Article

Clinical Significance of Hematological Indices for the Differential Diagnosis of Iron Deficiency Anemia and Thalassemia Trait

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Abstract

Background: The two most prevalent causes of microcytosis and hypochromic but have different causes and treatments, are iron deficiency anemia (IDA) and thalassemia trait (TT). Conventional diagnostic methods such as hemoglobin electrophoresis and Serum Ferritin are Reliable but may be costly and time-consuming, particularly in low resources. Therefore, Hematological Indices are CBC-derived tools that can be used for differentiation of IDA and TT.
Objectives: The present study assesses the diagnostic validity of several hematological indices for the distinction between TT and IDA.
Methods: A cross-sectional study was conducted on 171 patients who presented with microcytic anemia. IDA patients were confirmed through Serum Ferritin, whereas TT patients were diagnosed through Hb Electrophoresis between May 2024 and August 2024 in Test Zone Laboratory, Lahore, Pakistan, by convenience sampling technique. CBC parameters were analyzed on Sysmex XN 1000i. Patients diagnosed with Thalassemia trait and Iron deficiency anemia are included. Patients with recent blood Transfusion and those who are not confirmed IDA and TT patients are excluded.
Results: Significant differences were observed between IDA and TT groups in hemoglobin, RBC, MCV, MCH, and MCHC. Among All Srivastava Index performed the best with the highest diagnostic accuracy. Mentzer Kerman I also showed good results. In contrast, MDHL and Sirachainan showed poor availability.
Conclusion: Hematological indices stood out as the simple yet effective and cost-friendly method for the differential diagnosis of Iron deficiency Anemia and Thalassemia Trait. The Srivastava Index emerged as the reliable indices that can be effectively utilized during routine Clinical practice.
Keywords: Clinical Significance, hematological indices, iron deficiency anemia , thalassemia trait
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Introduction

Anemia is a condition marked by either inadequate levels of red blood cells or hemoglobin. Microcytic anemia characterizes a form of anemia where the mean corpuscular volume (MCV) measures below 80 mcm³ in adults. Iron deficiency stands out as the predominant acquired etiology of microcytic anemia.[1]The most typical congenital cause of microcytic Anemia is Thalassemia. It is an inherited blood disorder defined as unusual hemoglobin production that directly affects the oxygen-carrying capacity of RBCs.[2] In Thalassemia Trait which is also called as Thalassemia minor, there is one mutated gene that makes faulty hemoglobin in the blood but a carrier usually not possess any specific health issue. The most common Diagnosis is made through blood test, particularly Hb electrophoresis. Moreover there are some genetic testing that identifies the presence of Thalassemia mutation.[3] Cousin marriages are a common concept in Pakistan, which is the main reason that contributes approximately 5-8% of the carrier rate. In developing countries like Pakistan these genetic conditions becomes serious health issue, because people have less awareness about testing before marriages.[4] IDA and TT have similar symptoms but their treatment methods are different. Therefore it is necessary to provide accurate diagnosis to ensure effective treatment. Because of the same clinical symptoms, sometimes the differential diagnosis becomes difficult. Conventional diagnostic approaches, such as CBC and Serum iron levels and Hb electrophoresis, provide essential information but may not always clearly distinguish between the two conditions, and these are cost-effective and time-consuming.[5] In last few years, hematological indices

stand out as valuable a tool that improves the diagnostic accuracy of IDA and TT. These indices include Mentzer Index, MDHL Index, Pornprasert Index, Shine and Lal, England and Frazer, Ehsani Index, Sehgal Index, Matos and Carvalho and Sirachainan Index. These indices are calculated by using different formulas through CBC parameters. These Hematological indices provide quantitative information to clinicians or to laboratory Professionals about the erythropoietic processes which help differentiate IDA from TT. These indices takes less time than other traditional methods used to diagnose IDA and TT, thereby facilitating appropriate management. In 1922 Wintrobe introduced the terms Mean corpuscular Volume (MCV) that measures the size of RBC, Mean corpuscular hemoglobin (MCH) and Mean Corpuscular hemoglobin count MCHC that specify the hemoglobin measures in RBCs. By utilizing the values of hematocrit (packed cell volume), hemoglobin, and red blood cell count, indices can be computed. With the easy accessibility of electronic cell counters, red cell indices are now instinctively calculated as part of routine blood count assessments.[6]

The Mentzer Index (MI), created by Mentzer in1973, is the most often utilized. Lowered blood cell (RBC) counts and mean corpuscular volume (MCV) are the outcomes of reduced bone marrow production of red blood cells in iron deficiency anemia. Mentzer Index is calculate by MCV/RBC and has cut off value 13. When the value of Mentzer index is greater than 13, it indicates IDA while a value less than 13 suggest TT. However, Mentzer index don't have any unit calculated by using MCV and RBC. In thalassemia, the production of red blood cells is typically normal. However, due to a defective beta globin chain, the red blood cells become more fragile and smaller in size. As a result, individuals with thalassemia often exhibit a normal red blood cell count but have a lower mean corpuscular volume.[4]

Second is the Shine and Lal index(S&L), which was originally developed in 1973, Shine and Lal claimed that it could distinguish between TT and IDA patients. The cut off value of S&L is 1530. Less than 1530 indicates TT while above imply IDA. The formula of S&L is $MCV^2 \times MCH/100$. Some previous studies have proven that Shine and Lal index is one of the most effective indices among all in distinguishing Iron deficiency Anemia and Thalassemia Trait. The S&L indices also demonstrate strong performance in diagnosing mild to moderate and moderate to severe IDA cases.[7] The Srivastava index (SI) is helpful and practical for differentiating between IDA and BTT. The formula of Srivastava index is MCH/RBC. The cut off value of Srivastava Index is 3.8. The greater value than 3.8 suggest IDA whereas less value indicates TT.[8] Despite the presence of various Hematological indices, their diagnostic performance varies across different population for their clinical acceptability, that's why it is essential to evaluate the accuracy of all indices in local settings. In this study, we intended to assess the diagnostic accuracy and clinical utility of RBC indices for differential diagnosis of thalassemia trait (TT) and iron-deficiency anemia (IDA).

Methods

This cross-sectional analytical study was conducted at Hussain Memorial Hospital and Test Zone Laboratory, Lahore, Pakistan, from May to August 2024. With an expected sensitivity of 85%, a 95% confidence level, and a 5% margin of error, the sample size was determined to be at least 162. There were 171 patients in all in which there are 92 patients of Thalassemia trait and 79 patient of Iron deficiency anemia that are included in the study.

The Sample Size was determined by a non-probability convenience sampling technique. Venous Blood samples were taken from all the Patients already diagnosed with iron deficiency anemia and thalassemia trait, confirmed by serum iron studies and hb electrophoresis, and were taken in EDTA vials. Complete Blood Count was performed. Hematological indices including Mentzer and Srivastava, are derived indices, all were calculated from CBC parameters by using Mathematical formulas.

The cut-off values for each index were adopted from previously published literature. Values below or above the defined thresholds were used to classify patients as TT or IDA accordingly as in Table 1.

Patients with a history of acute and chronic infections, diseases that affect erythropoiesis, recent blood transfusions, and any other known hematological disorders other than TT or IDA were excluded from the study. However, patients aged 18-60 years of both

genders with a confirmed diagnosis of IDA and TT were included in the study. Informed Consent was taken from all participants before taking the Sample. This study was approved by the Ethics Committee of Hussain Memorial Hospital and Test Zone Laboratory with ERC reference numbers HCHS/ 2024/ERC/74 and 24/52, respectively.

The Instrument used for CBC was the Sysmex XS 1000i. The Sysmex XS-1000i Hematology Analyzer operates on the principles of Fluorescent Flow Cytometry and Hydrodynamic Focusing to achieve precise differentiation and counting of blood cells. Hemoglobin analysis is performed using a cyanide-free reagent, Sodium Laurel Sulfate (SLS), ensuring no interference from high WBC counts, lipemia, or abnormal proteins. Hematocrit is measured by summing the pulse heights of all RBC counts, providing an accurate measure based on cell volume. Complete blood count abnormalities were defined as the elevation and reduction from the mentioned ranges of the following CBC parameters: Hemoglobin Count (Males13.5-17.5, Females11.5-15.5), Red Blood Cell Count (Males 4.5-6.5, Females3.9-5.6), MCV (80-95fL), MCH (27-34pg), MCHC (32-36g/dl) and RDW (11-15%).

Statistical Analysis:

The data was analyzed using the SPSS statistical software version 25. Frequencies and Percentages are calculated for categorical variables. Continuous Variables were tested by using the Kolmogorov-Smirnov test, as the data were not normally distributed ($p < 0.05$), results were presented as median (IQR). The Mann-Whitney U Test was used to compare continuous variables in the IDA and TT groups. The U-statistic, P value, and effect size were calculated for comparison. The Receiver Operating Characteristic (ROC) curve analysis was performed for each RBC index to assess the diagnostic accuracy of hematological indices. Area Under the Curve (AUC) with 95% confidence intervals was calculated. Optimal cut-off values were determined using Youden's Index. Two tailed were used, and a p-value less than 0.05 was considered statistically significant.

Table 1: *Formulas and Cut-off Values of Red Cell Discrimination Indices*

Discrimination Indices	Calculation	Cut-off (TT)	Cut-off (IDA)
RBC	RBC	>5	<5
Mentzer	MCV / RBC	<13	>13
England and Fraser(E&F)	MCV−RBC−(5 × HB)−3.4	<0	>0
ShineandLal(S&L)	MCV×MCV×MCH / 100	<1530	>1530
Srivastava	MCH/RBC	<3.8	>3.8
Sirdah	MCV−RBC−(3× Hb)	<27	>27
Ehsani	MCV−(10× RBC)	<15	>15
MDHL	(MCH×RBC) / MCV	>1.63	<1.63
Pornprasert	MCHC	<31	>31
KermanI	(MCV×MCH) / RBC	<300	300–400
Sehgal	MCV^2 / RBC	<972	>972
MatosandCarvalho(MC)	1.91× RBC+0.44× MCHC	>23.85	<23.85
Sirachainan	1.5× HB – 0.05 × MCV	>14	<14

RBC: Red Blood Cell count; MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Hemoglobin; MCHC: Mean Corpuscular Hemoglobin Concentration;
Hb: Hemoglobin. Cut-off values were adopted from previously published literature and applied uniformly to classify cases as iron deficiency anemia (IDA) or thalassemia trait (TT).

Results

Demographic Characteristics of the Study Population:

A total of 171 confirmed diagnosed patients were included in the study, among them 79 patients are serum ferritin-based confirmed Iron Deficiency Anemia patients, and 92 patients are Hb electrophoresis-based confirmed Thalassemia trait patients, as presented in Figure 1.

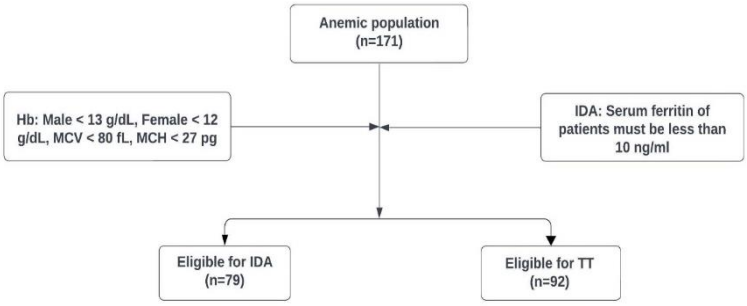

Figure 1: *Study Design. Hb: hemoglobin; MCV: mean corpuscular volume; MCH: mean*

In IDA, male patients were 2% (N=4) while female patients were 44% (N=75). In TT, male patients were 27% (N=46) while female patients were 27% (N=46), as shown in Figure 2.

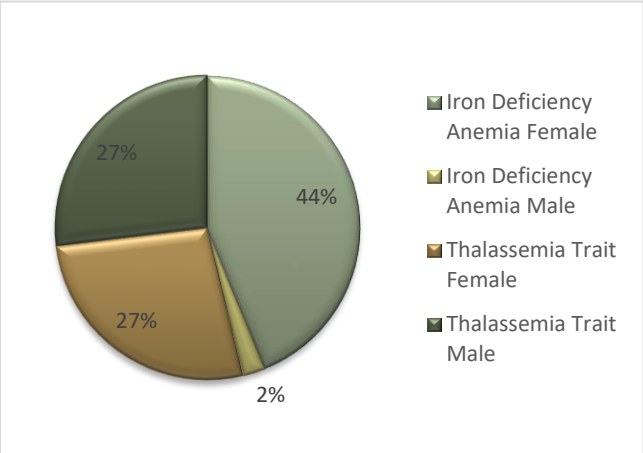

Figure 2: *Gender Distribution*

In IDA, the mean age of the participants was approximately 19.7 years, while in TT, the mean ageof the participants was approximately 23 years.

The age group that was most affected by IDA and TT was 16-30, and the age group that was least affected by IDA and TT was 51-65, as shown in Figure 3.

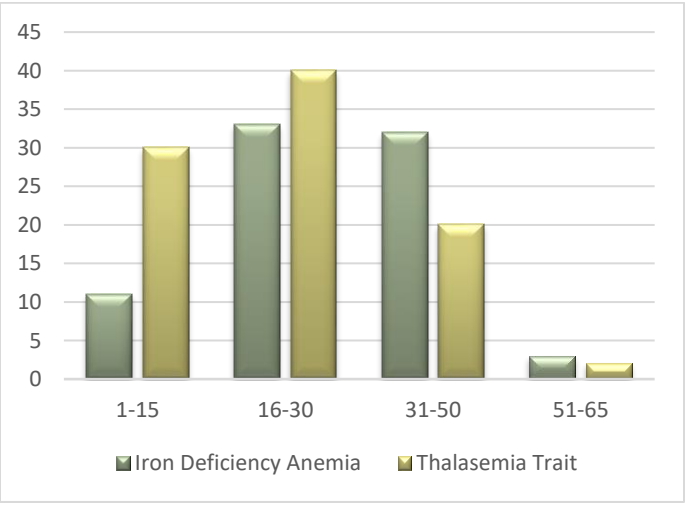

Figure 3: *Age wise distribution for IDA and TT*

Laboratory data of IDA and TT patients included Age, Hemoglobin Count, RBC count, MCV Count, MCH Count, and MCHC Count. Because the Kolmogorov-Smirnov test indicated non-normal distribution of Laboratory Parameters ($p < 0.05$), results are expressed as median (Interquartile range). A two-tailed Mann-Whitney U test ($\alpha = 0.05$) was used to perform Comparisons between the TT and IDA groups. Significant differences were observed between TT and IDA for Hb, RBC, MCV, MCH, and MCHC (all $p < 0015$), and are presented in Table 2.

Table 2: *Laboratory Parameters and Age, Mean Ranks and Test Statistics with Mann-Whitney U Test*

Laboratory Parameters	Median± IQR	Group	Mean Rank	U value	P value
Age	25.0±17.3	TT	92.24	3059.92	<0.001
		IDA	62.37		
Hb	10.4 ± 2.1	TT	76.08	4546.64	0.015
		IDA	94.52		
RBC	5.0 ± 1.4	TT	44.88	7417.04	<0.001
		IDA	121.31		
MCV	70.0 ± 13.6	TT	105.25	1863	<0.001
		IDA	69.47		
MCH	20.0±5.7	TT	115.32	936.56	<0.001
		IDA	60.83		
MCHC	29.4± 4.8	TT	117.25	759	<0.001
		IDA	59.16		

Data was not normally distributed according to the Kolmogorov-Smirnov test ($p < 0.05$). Therefore, compar-isons were performed using the two-tailed Mann-Whitney U test with $\alpha = 0.05$

Sensitivity and Specificity of Indices

In this study, Thalassemia Trait (TT) was considered the positive condition for sensitivity analysis. The diagnostic performance of every red blood cell index was assessed for the correct detection between IDA and TT. If we talk about RBC count, the sensitivity was higher among TT patients, but the patients with IDA showed greater specificity. Regarding the Mentzer Index, the sensitivity was higher, but the specificity was lower when compared to the TT patient group. However, the England and Frazer index appeared to have high sensitivity in IDA, while the patients with TT reported high specificity. The analysis of Shine and Lal indicated that TT patients had greater sensitivity but lower specificity when compared with the IDA group. For the Srivastava Index, IDA patients exhibited higher sensitivity, whereas TT patients had greater specificity. A similar pattern was observed in the Sirdah index as well, in which IDA patients had low specificity but higher sensitivity relative to TT patients. Concerning the Ehsani Index, IDA patients revealed higher sensitivity, while TT patients showed higher specificity, while TT patients group showed greater specificity. The MDHL Index, which is abbreviated as Mean Density hemoglobin per Liter of Blood, TT patients showed greater sensitivity but lower specificity with comparison to IDA patients. The Pornprasert indicates higher sensitivity in TT patients, while IDA patients had greater specificity. The Kerman I Index proclaimed higher specificity and lower sensitivity in IDA patients as compared to TT patients. The Sehgal index showed higher sensitivity for TT patients with

respect to IDA patients that demonstrated higher specificity. In the final, the Matos and Carvalho Index expressed that TT patients had higher sensitivity but lower specificity. The Sirachainan Index showed similar, higher sensitivity and lower specificity in IDA patients when compared with TT patients.

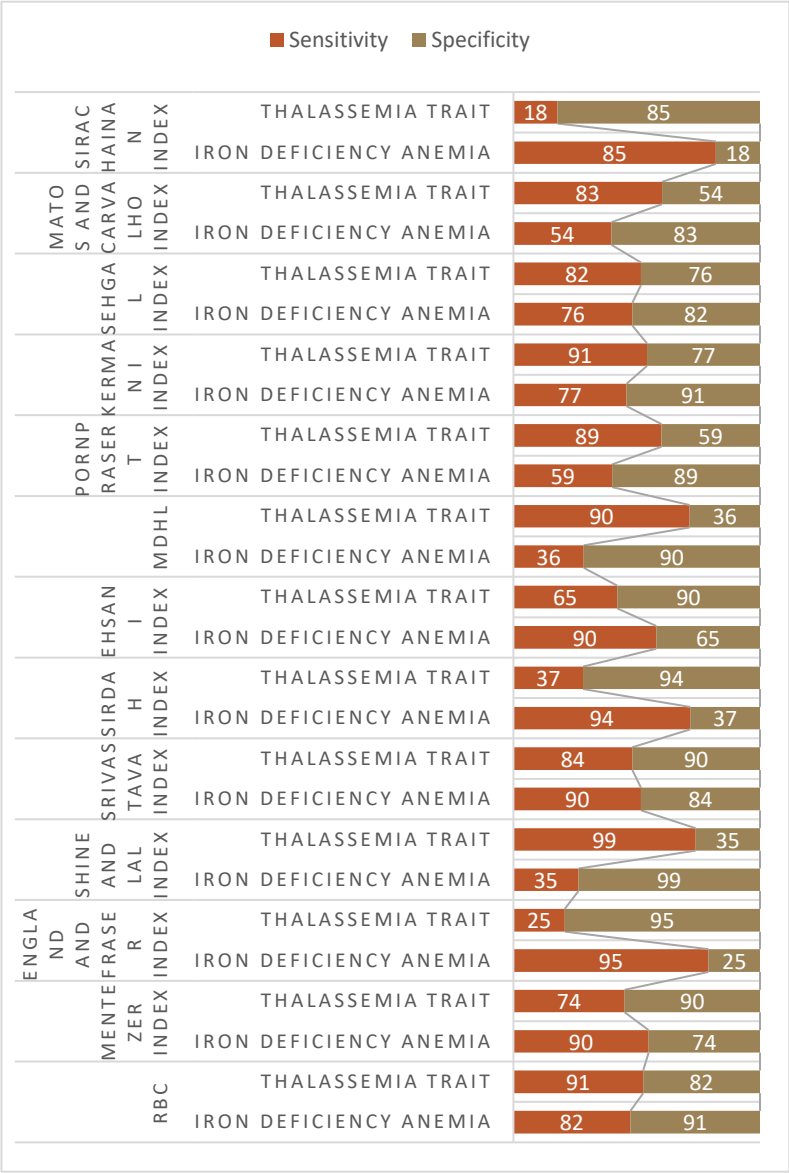

Figure 4: *Sensitivity and Specificity of Hematological Indices*

The RBC for TT shows 84 true positives, 14 false positives, 8 false negatives, and 65 true negatives, with a total of 149 correctly classified cases. Similarly, RBC for IDA shows 65 true positives, 8 false positives, 14 false negatives, and 84 true negatives. Other indices like Mentzer, England and Fraser, Shine and Lal, and others follow the same structure. To rank these indices based on their diagnostic performance, we use the total number of correctly diagnosed patients for each index. Here is the ranking from highest to lowest performance: RBC > Srivastava > Kerman I > Mentzer > Sehgal > Ehsani >Pornprasert> Shine and Lal > Matos and Carvalho > Sirdah > MDHL > England and Fraser >Sirachainan. This ranking provides a clear view of which indices have the highest performance in correctly diagnosing patients with TT and IDA. The True Positive Rate represents the proportion of actual positives correctly identified, also known as Sensitivity. The True Negative Rate indicates the proportion of actual negatives correctly identified, reflecting Specificity. The False Positive Rate is the proportion of negatives incorrectly identified as positives, while the False Negative Rate is the proportion of positives incorrectly identified as negatives. Additionally, the Positive Predictive Value is the proportion of positive test results that are true positives, whereas the Negative Predictive Value is the proportion of negative test results that are true negatives.

Based on performance metrics, the discriminant formulas ranked from highest to lowest diagnostic performance as follows: RBC > Srivastava > Kerman I > Mentzer > Sehgal > Ehsani >Pornprasert> Shine and Lal > Matos and Carvalho > Sirdah > MDHL > England and Fraser >Sirachainan as shown in Table 3.

Table 3: *Diagnostic Ability of Hematological Indices*

Hematological Indices	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)	Youden Index (%)	p-value
Srivastava	83.69	89.87	90.59	82.56	86.5	73.56	<0.001
Mentzer	73.91	89.87	89.47	74.74	81.2	63.78	<0.001
Kerman I	91.30	77.22	82.35	88.41	84.7	68.52	<0.001
Ehsani	65.22	89.87	88.24	68.93	76.6	55.09	<0.001
Sehgal	81.52	75.95	79.79	77.92	78.9	57.40	<0.001
Pornprasert	89.13	59.49	71.93	82.46	75.4	48.62	<0.001
Sirdah	36.96	93.67	87.18	56.06	63.1	30.63	<0.001
England and Fraser (E&F)	25.00	94.94	85.19	52.08	57.3	19.94	<0.001
RBC	91.30	82.28	85.71	89.04	87.1	73.58	<0.001
Shine and Lal (S&L)	98.91	35.44	64.08	96.55	69.5	34.35	<0.001
Sirachainan	18.48	84.81	58.62	47.18	49.1	3.29	0.040
Matos & Carvalho	82.61	54.43	67.86	72.88	69.5	37.04	<0.001
MDHL	89.87	35.87	54.62	80.49	60.8	25.74	<0.001

PPV: Positive Predictive Value; NPV: Negative Predictive Value

Diagnostic Ability of Hematological Indices:

Receiver Operating Characteristic (ROC) curve analysis was performed for each Hematological Index. The Srivastava Index demonstrated the highest overall diagnostic performance, followed by the Mentzer and Kerman I Indices, presented in Table 4.

Table 4: *Final Ranking; Diagnostic Performance of Hematological Indices Based on AUC with 95% Confidence Intervals*

Rank	Index	AUC (95% CI)	Diagnostic Performance	p-value
1	Srivastava Index	0.937 (0.902–0.972)	High	< 0.001
2	Mentzer Index	0.897 (0.852–0.942)	High	< 0.001
3	Kerman I Index	0.894 (0.848–0.940)	High	< 0.001
4	Ehsani Index	0.892 (0.846–0.938)	High	< 0.001
5	Sehgal Index	0.862 (0.809–0.915)	High	< 0.001
6	Pornprasert Index	0.840 (0.782–0.898)	Moderate	< 0.001
7	Sirdah Index	0.832 (0.772–0.892)	Moderate	< 0.001
8	England & Fraser Index	0.830 (0.770–0.890)	Moderate	< 0.001
9	RBC Index	0.770 (0.702–0.838)	Moderate	< 0.001
10	Shine & Lal Index	0.764 (0.694–0.834)	Moderate	< 0.001
11	Sirachainan Index	0.346 (0.266–0.426)	Poor	0.040
12	Matos & Carvalho Index	0.286 (0.212–0.360)	Poor	< 0.001
13	MDHL Index	0.217 (0.153–0.281)	Poor	< 0.001

Indices were categorized as high (AUC ≥ 0.85), moderate (AUC 0.70–0.84), and poor (AUC < 0.60) diagnostic performance. AUC: Area Under the Curve; CI: Confidence Interval.

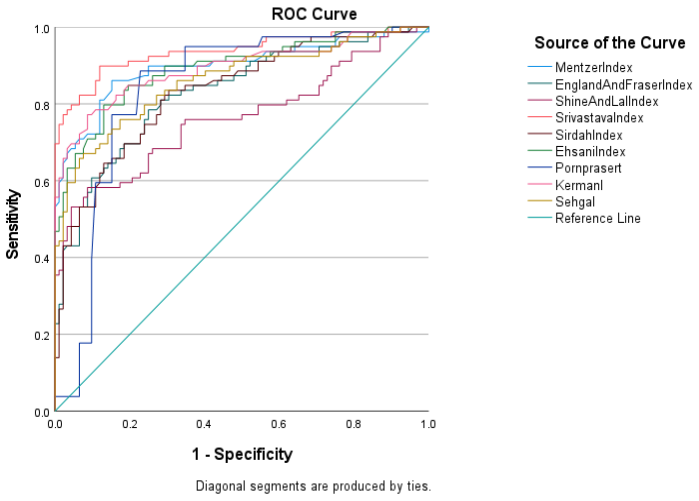

Figure 5: *ROC Curve Analysis*

Good to exceptional discrimination capacity of hematological indices for IDA from TT is demonstrated by the Mentzer Index (0.897), Srivastava Index (0.937), Ehsani Index (0.892), Kerman I (0.894), and Sehgal (0.862), with a high AUC (>= 0.85) (P value <0.001). Low AUC (<0.60), MDHL (0.217), Matos & Carvalho (MC) (0.286), and Sirachainan (0.346) as presented in Figure 5. This ranking highlyghts the importance of each formula's diagnostic performance. Based on the metrics, the discriminant formulas ranked as follows: Srivastava > Mentzer > Kerman I > Ehsani > Sehgal > Pornprasert > Sirdah > England & Fraser > RBC > Shine & Lal > Sirachainan > Matos & Carvalho > MDHL.

Discussion

IDA and TT are the most common causes of microcytic anemia, and these two conditions typically show similar clinical symptoms. The confirmed diagnosis is shown based on the HbA2 increase in the case of TT[8,9], but in the case of IDA, it shows a decrease in Serum ferritin.[10] The accurate discrimination between these two anemias is vital. Previous research has highlighted the prevalence of both hematological disorders and utilized several hematological indices to evaluate their diagnostic performance in distinguishing between IDA and TT, but none of the indices showed 100% diagnosis in various studies.[11, 12] In this study, we examined 171 confirmed diagnosed patients, of whom 79 were IDA patients, and 92 were confirmed TT patients. IDA patients were confirmed by Ferritin test, whereas TT patients were confirmed by Hb electrophoresis. In our study, we found that there is a remarkable difference in gender distribution between the two conditions. In IDA, there were more female patients (94.9%) while only 5.1% were male. However, some past studies have found that IDA is more prevalent in females due to various reasons, such as pregnancy and menstruation, which ultimately leads to loss of iron.[13] On the other hand, TT may affect both genders because it is a genetic disease.[14]

The mean age for IDA patients is approximately 19.7 and 23 years for the TT patient group. In our study, both conditions primarily affected the age group of 16-30years, while the least affected group was 51-65 years. Most studies indicate that IDAs are common in younger populations, possibly due to dietary habits.[13] Laboratory data showed significant differences between IDA and TT patients in terms of Hemo-

globin (Hb) count, red blood cell (RBC) count, Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), and Mean Corpuscular Hemoglobin Concentration (MCHC). All these parameters showed a p-value less than 0.05, that indicate the difference observed were statically substantial. IDA and TT patients had lower hemoglobin levels and lower RBC count. In an IDA patient's body is unable to make sufficient hemoglobin due to a lack of iron. In TT, due to ineffective erythropoiesis, the body struggles to provide enough red blood cells, and this leads to uneven iron content.[15]

The significant purpose of this study is to evaluate the accuracy and effectiveness of hematological indices that are helpful to differentiate between IDA and TT. Mina Jahangiri et al. conducted research that added sensitivity and specificity analysis and compared these matrices with the AUC.[12] ROC curve analysis was also done to compare the diagnostic accuracy by using the area under the curve. Srivastava Index revealed the highest diagnostic performance, showing the sensitivity of 83.69% and 89.7% specificity with an AUC of 0.937. These finding indicates that the Srivastava index showed the highest performance in differentiating IDA and βTT. Mentzer Index showed a good balance between the sensitivity and specificity, 73.91% and 89.87%, respectively. As compared to the Mentzer Index, the Kerman I index showed higher sensitivity than specificity, with a TPR 91.30% and TNR of 77.22%. Its AUC value is 0.894, showing that it has strong diagnostic performance. Ehsani Index had a sensitivity 65.22% and a specificity of 89.7% with an AUC of 0.892. The Sehgal Index indicates good diagnostic performance with a sensitivity of 81.52% and specificity of 75.95%, in the results, the AUC is 0.862. Pornprasert displayed high sensitivity but moderate specificity with TPR 89.13% and TNR of 59.49%, resulting AUC value of 0.840. The AUC value of the Sirdah Index is 0.832 with low sensitivity 36.96% but high specificity 93.67% that means this index can correctly diagnose the true negatives. Similar to the Sirdah Index, England and Fraser Index also showed less sensitivity 25% and specificity 94.94% 0.830. RBC Index showed balanced performance with an AUC of 0.770. RBC index is considered an effective index but not the highest, showing a sensitivity of 91.3% and a specificity of 82.28%. Shine and Lal Index had limited diagnostic performance because of low specificity 35.44% even though the sensitivity is high 98.91% with an AUC of 0.764. Sirachainan Index showed sensitivity of 18.48% and Specificity of 84.81% with a low AUC value of 0.346. The Sirachainan showed an AUC value of 0.346 with poor diagnostic performance. Matos and Carvalho also showed poor diagnostic performance with a sensitivity 82.61% and a specificity 54.43%. MDHL showed an AUC of 0.217. Despite its high sensitivity, it shows limited diagnostic ability. According to Madan et al., the Shine and Lal Index stood as the best indices.[16] As compared to our studies, Srivastava and Mentzer indices emerged as the as the top notch indices. The study conducted by Mina Jahangiri showed the same results.[12] This analysis involves different factors of measures, including error rates, predictive values, likelihood ratios, Accuracy and diagnostic odds ratio. The RBC demonstrates a high diagnostic performance with a Youden's Index of 73.58% DOR of 48.73 and accuracy of 87.1%. Mentzer Index shows Youden's Index 63.78%, accuracy 81.2%and DOR of 25.13.In a study by Vehapoglu et al., the Mentzer Index considered as the most reliable index.[17] The England and Frazer Index showed accuracy 19.94% with low Youden's Index and DOR of 6.25 which ultimately proves that it has limited diagnose ability. Unlike to Amar Wahan et al., whose study concluded that this index diagnosed 90% of the individuals correctly.[18] The Shine and Lal Index offers Youden's Index 34.35%, accuracy 69.5% and a high DOR of 49.81 showing it moderate effectiveness. In contrast the research of Amer Wahan et al. showed 55% of the reliable result.[18] A study conducted in Rawalpindi found out that Srivastava Index identifies 90.25% of the patients.[19] RBC formula shows a high Youden's Index of 73.56% an accuracy of 86.5% and DOR of 45.52. The Sirdah formula has moderate formula with Youden's Index of 30.63% accuracy of 63.1% and DOR of 8.68. The Ehsani formula has Youden's Index 55.09%, accuracy of 76.6% and DOR of 16.64.[20] The Youden's Index of MDHL Index 25.75% accuracy of 60.8% and DOR of 4.96. The Pornprasert shows Youden's Index of 48.62%, accuracy of 75.4% and DOR of 12.04. The research conducted by frontiers of pediatricians, showed same 50% diagnostics accuracy of Pornprasert Index.[21] The Kerman I Index showed Youden's Index 68.52% accuracy 84.7% and DOR of 35.57. Sehgal Index shows moderate performance. Matos and Carvalho displayed

Youden's Index 37.04% accuracy of 69.5% and DOR 5.67. Sirachainan showed least diagnostic performance. In Summary Indices with AUC values ≥ 0.75 ($P < 0.001$), specifically Srivastava Mentzer, Kerman I and Ehsani Index Showed the best in differentiating IDA and TT.

Conclusion

This cross- sectional study highlights that several hematological indices can be used to differentiate between Iron Deficiency Anemia and Thalassemia Trait, as an alternative to costly test. Srivastava index stood out as the best performing index while Mentzer Index also performed well. Some Indices like Shine and Lal despite high sensitivity expressed limited diagnostic performance. These indices serve as valuable tool for clinicians to accurately and timely diagnose these common microcytic anemias.

Ethical Approval: The Research & Ethics Committee, Hussain College of Health Sciences approved this study vide Ref No. HCHS/ 2024/ERC/74.

Conflict of Interest / Disclosure: Nil.

Funding Source: Nil.

Authors' Contribution:

FK: Conception and design; acquisition, analysis and interpretation of data; drafting of article, critical revisions for important intellectual content, final approval of the version to be published

BF: Acquisition of data; drafting of article.

BM: Analysis and interpretation of data

MN: drafting of article, critical revisions for important intellectual content

MM: Analysis and interpretation of data, critical revisions for important intellectual content

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