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ORIGINAL ARTICLE

Significance of New Index Matos and Carvalho Index in differentiation of iron deficiency anemia and beta thalassemia trait.

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ABSTRACT… Objective: To assess the value of the Matos and Carvalho index (MCI) in detecting beta thalassemia traits in patients with microcytic hypochromic anemia. **Study Design:** Retrospective study. **Setting:** Department of Hematology, CHK Central Laboratory Dr Ruth KM Pfau Civil Hospital Karachi, Pakistan. **Period:** 1st July to 31st December 2022. **Methods:** All newly diagnosed cases of iron deficiency and Beta Thalassemia trait were included. 297 patients with an age range of 1–60 years were evaluated. We calculated 4 discrimination indices in all patients with hemoglobin (Hb) values of <10 g/dL. The patient groups were evaluated according to red blood count; the Mentzer index (MI); the Srivastava index (SI) and Matos and Carvalho index (MCI). In this study complete blood counts for red cell indices were processed on a Hematology analyzer (XN-1000), HPLC was done on Arkray HA-8108T and serum ferritin was done on a chemistry analyzer (COBAS 501). **Results:** 297 patients were included in the present study with hypochromic, microcytic anemia for beta thalassemia trait (BTT) and iron deficiency anemia (IDA). HPLC detected 141 patients with BTT, while 156 were diagnosed with IDA. The average age of Patients with BTT was 14.5 years, whereas the average age of IDA patients was 20.6 years. The Mentzer index correctly identified patients at 64.6%, followed by MCI and SI at 63.2%, 62.2%, and 55.2%, respectively. **Conclusion:** The Matos & Carvalho Index offers an easy and accessible way to enhance thalassemia detection and iron deficiency anemia by the simple test of CBC at the frontline. In this study Srivastava index (SI) demonstrated the most robust reliability levels when distinguishing between beta thalassemia trait and Iron deficiency anemia. However, the absence of red cell indices and methods that possess 100% specificity, efficacy, and sensitivity hinders the differentiation of BTT from other hypochromic microcytic anemia.

Key words: Anaemia, Beta Thalassemia Trait, Ferritin, HPLC, Microcytic, Matos and Carvalho Index.

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INTRODUCTION

Thalassemia was first described by Dr. Thomas Cooley in the pediatric population of Mediterranean region almost a century ago.[1] most of the thalassemia major patients have severe anemia in the initial 2 years of life and without treatment and transfusion, they have shortened life expectancy.[2]

According to literature 5%–7% cases reported in the population of the world, which has carrier state of beta thalassemia. . It involves genetic Hb variations in the thalassemia.[3]

According to reported literature around 800 million cases of anemia, major cases of anemia are secondary to iron deficiency anemia or thalassemia carrier state. World Health Organization (WHO) estimated that iron deficiency anemia can cause 273,000 deaths, and developing countries are majorly affected around 97%.[4]

In a developing country like Pakistan, this hemoglobin disorder can cause burden on the health care system because of regular and frequent blood transfusions and treatment of complications such as iron overload are quite costly, mostly affected persons and their family cannot afford the treatment.[5]

Pakistan is one of those countries with the highest thalassemia burden.[6] According to safe blood transfusion authority, about 2.7 million blood collections are made in Pakistan annually, around one fourth blood products are used for the treatment of thalassemia.[7] This is quite a large figure of blood transfusions for a developing country like Pakistan.

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The most common causes of microcytic, hypochromic anaemia are iron deficiency anemia (IDA) and beta thalassemia trait (BTT), especially in the pediatric population.[8]

In the Southeast Asia, the Mediterranean region, middle east and central Africa, around 50% of the population having thalassemia trait.[9]

Thalassemia carriers can be discovered in people with no obvious signs and symptoms. To confirm the diagnosis of thalassemia trait, we need specialized tests such as hemoglobin electrophoresis or high-performance liquid chromatography (HPLC). In the patients who present with hypochromic, microcytic picture on CBC, serum iron profile and HPLC should be done for exact diagnosis. Diagnosis of the Beta thalassemia trait (TT) is usually confirmed by hemoglobin electrophoresis or HPLC method with elevated HbA2 levels (>3.5%).

In previous studies, several red cell indices parameters were found to be useful to simplify the difference between iron deficiency anemia and thalassemia carrier state-based complete blood count parameter analysis. The formula developed by Green & King [MCV2) × RDW]/(Hb × 100) where MCV means corpuscular volume, RDW is RBC distribution width and Hb is hemoglobin} is a useful formula according to some studies. The major disadvantage is that in the formula the RDW has to be calculated which is not mentioned in CBC analysis reports. In addition, the current indexes that have been recorded in literature lack the biology molecular studies to diagnose thalassemia. This analysis is essential to confirm the diagnosis and exclude concurrent diseases. A novel modified index, called the Matos and Carvalho index (MCI)[10] has been reported in recent studies. This index utilizes the RBC count and mean corpuscular hemoglobin concentration (MCHC) parameters. The formula MCI is = (1.91 × RBC) + (0.44 × MCHC). Through the analysis of the ROC curve> MCI has demonstrated a discriminative cut-off value of 23.85 to distinguish between Iron Deficiency Anemia (IDA) and Beta Thalassemia (BTT). Patients with an index value less than 23.85 are categorized as having Iron Deficiency Anemia, while those with an index value greater than 23.85 are identified as carriers of Thalassemia. This research aims to assess the significance of a new index capable of distinguishing between Iron Deficiency Anemia and carriers of Thalassemia. This will be accomplished with simple red cell indices provided by all automatic hematology analyzers.

METHODS

This retrospective cross sectional study was conducted in the Hematology Department CHK Central Laboratory Dr Ruth KM Pfau Civil Hospital Karachi from 1st July to 31st December 2022.

Ethical approval was obtained from the Institutional Review Board (IRB-2953/DUHS/EXEMPTION/2023/134). CBC, serum ferritin levels, Hb A2 and detection of Hb H inclusion bodies were done to confirm the diagnosis. All newly identified cases of beta thalassemia trait and iron deficiency anemia were included based on serum ferritin and CBC results. In samples obtained in our laboratory, a complete blood count was performed on an XN 1000 analyser, followed by a ferritin analysis on a COBAS and HPLC by Arkray ADAMs HA-8108T was used.

Over 6 months, 297 patients with confirmed microcytic anaemias were included with non-probability sampling in the study. 156 patients were diagnosed with iron deficiency anemia based on low hemoglobin, low MCV and MCH levels and low serum ferritin levels. 141 patients were diagnosed with beta-thalassemia trait based on low Hb and MCV levels, normal serum ferritin and elevated levels of Hb A2 (>3.5%).

Patients were classified as having BTT if their HbA2 level was greater than 3.5% or IDA if their blood ferritin level was less than 10 ng/ml.

In this research article, five discrimination indices were calculated and used for evaluation. These indices included specificity, sensitivity, positive predictive value (PPV), negative predictive value (NPV), and Youden's index. The values for each discrimination index were analyzed and compared to the ones reported in the previous research reports. Specifically, the Mentzer index (MI), the Srivastava Index (SI), the Matos & Carvalho index (MCI), and

the red blood cell (RBC) count were analyzed. The data were presented as the mean ± standard deviation. Data analysis was performed using the SPSS version 24.0 program. A P-value of < 0.05 was considered statistically significant.

RESULTS

Two hundred nine seven patients of microcytic, hypochromic anemia were enrolled and on the screening of these cases, they diagnosed iron deficiency anemia (IDA) and beta thalassemia trait (BTT). 141 were diagnosed with BTT using HPLC, while 156 were diagnosed with IDA based on low ferritin levels. It was observed that patients with BTT had a mean age of 14.5 years, whereas patients with IDA had a mean age of 20.6 years.

In the Table-I red cell indices formula mentioned, were applied to distinguish between IDA and BTT in our study, such as RBC count, Mentzer index (MI), Matos & Carvalho (MCI), and Srivastava index (SI).

In this study, the mean and ranges of complete blood count (CBC) parameters were calculated in the present study. The comparison of discrimination indices for both study groups are shown in Table-II.

In our study, the most reliable index for identifying the IDA and BTT patients at a rate of 64.6%, followed by RBC count, MCI, and SI at rates of 63.2%, 62.2%, and 55.2%, respectively, which is shown in Table-II.

Sensitivity, specificity, positive predictive value, negative predictive value, and Youden's index of each discrimination index mentioned in Table-III. The most sensitive index for BTT was the Srivastava index (SI) with sensitivity, specificity and Youden's index of 70.9%, 77.8% and 48.1% respectively, followed by the Mentzer index (MI), RBC count and Matos & Carvalho index (MCI). The MCI index showed great sensitivity and specificity for iron deficiency anemia cases (98%) & (92.1%) respectively, followed by MI, RBC count and SI index.

The mean Matos & Carvalho Index (MCI) was 24.3 in patients with BTT and 21.4 in those with IDA, with a notable P-value of 0.001.

TABLE-I

Different RBC indices and mathematical formulas used to differentiate between -TT and IDA

Hematological Index	Formula
Mentzer index (MI)	MCV/RBC
MCI	(1.91×RBC) +(0.44×MCHC)
Srivastava Index (SI)	MCH/RBC

TABLE-II

Results obtained from each discrimination index.

Indices (Cut off)	IDA (156)	BTT (141)	Correctly Diagnosed Subjects	Correctly Diagnosed (%)
MCI				
IDA <23.85	153	109	185	62.2
TT >23.85	03	32		
Mentzer				
IDA >13	120	69	192	64.6
TT <13	36	72		
SI				
IDA >4.4	64	41	164	55.2
TT <4.4	92	100		
RBC count				
IDA <5.0	116	69	188	63.2
TT >5.0	40	72		

DISCUSSION

Several studies have established different indices or formulas based on red blood cell (RBC) indices that can be utilized for the differentiation of iron deficiency anaemia (IDA) from beta thalassemia trait (BTT). The differentiation between these two conditions of microcytic anemia remains a challenge for physicians or hematologists because correct diagnosis is needed for the management and prevention of the disease.

In the present study, a new index (MCI) was used to validate in the screening of IDA and BTT as reported in the previous study by Matos. Our research suggests that MCI may not serve as the most reliable indicator for physicians in detecting beta thalassemia traits.

TABLE-III

Sensitivity, specificity, positive predictive value, negative predictive value, and Youden's index of each discrimination index

Index	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Efficiency	Youden's indices
RBCx10^12/L						
IDA	74.3	84.9	82.2	77.9	79.8	59.2
TT	51	75	46.1	79.3	68.7	26.9
MI						
IDA	76.9	87	85.1	79.6	82	64
TT	51	75	46.1	79.3	68.7	26.9
MCI						
IDA	98	92.1	92.7	97.9	95	90
TT	22.7	68.1	20.5	70.8	56	-9.2
SI						
IDA	41	64.6	45.3	60.5	54.8	5.6
TT	70.9	77.8	64	82.7	75.3	48.1

No red cell index or formula offers complete sensitivity, specificity, and efficacy (100.0%) in distinguishing BTT from IDA. The diversity of thalassemia mutations significantly influences these variations.

The RBC count emerges as a readily accessible and straightforward method for BTT detection. Through comprehensive statistical analysis to effectively distinguish between IDA and thalassemia traits. Advanced or special investigations such as High-performance liquid chromatography (HPLC) may not be available or affordable in healthcare facilities, especially in developing countries.

The Physician must know these indexes that can help in the differentiation between BTT and IDA. In this study, we aimed to identify more effective RBC indices and formulas that are more suitable for our specific circumstances in comparison to previous studies. The accuracy rate of correctly identifying patients' MCI values was found to be 62.2%, which is noticeably lower than the 99.6% reported in Matos et al.'s study.[11] It is important to acknowledge the limitation of using the MCI index as a screening test for BTT; this constraint was previously mentioned in Hoffmann's study. Hoffmann proposed that the utilization of the MCI index may be more appropriate following additional justification based on the patient population.[12]

According to the data available in our study, the Srivastava index, exhibited a higher value than MCI in identifying patients with β-thalassemia trait (BTT). This index also demonstrated the highest Youden's index of 48.1% in accurately distinguishing between the β-thalassemia trait (BTT) and iron deficiency anemia (IDA). The calculation of the Mentzer index correctly diagnosed 64.6% of patients with microcytic anemia, which is comparable to findings from other studies.[13-14]

This formula exhibited a substantial level of sensitivity and specificity. Moreover, the MCI demonstrated a high positive predictive value (PPV) of 92.7%, which indicates its suitability as a screening tool in clinical practice. Notably, the MCI displayed a high sensitivity of 90% in detecting IDA. Given that sensitivity signifies the proportion of individuals correctly identified with the iron deficiency, the MCI's high sensitivity in detecting IDA suggests that it is a valuable tool for identifying the iron deficiency cases.

Ebrahim reported that cell differential counters help in the diagnosis process by enabling easy determination of RBC count.

Another study in the Indonesian population especially Surabaya also showed that the Matos and Carvalho Index has a low performance in discriminating BTT and IDA. Subjects used in the study were aged 3-17 years with different hematological profiles than the adult population (> 12 years) used in this study. Some hematological profiles in children tend to have lower values than adults affecting the study results.[15]

In our study, the RBC count and MI index showed more sensitivity for iron deficiency cases than BTT. RBC count and MI index showed sensitivity for IDA 74.3% and 76% respectively.

Previous studies, such as Fakher et al study (82%), Vehapoglu et al. study (65.3%), and George et al study (63.4%), have reported RBC indices with high Youden's indices.[16-18] This is supported by

the hematological profile between populations in Iran and Indonesia which both have higher average RBC values. In addition to the Matos and Carvalho indices, other indices in this study have also been shown to produce varying results compared to previous studies within sample populations globally or within the same geographical region.[19]

The spectrum of mutations could be the potential as a contributing factor to the differences in study outcomes, given the prevalence of thalassemia mutations in the global population, including Indonesia. This has been demonstrated in studies on the spectrum of beta-thalassemia mutations in Central Java and West Java.[20-22]

CONCLUSION

Our study suggests that MCI may not be the most reliable indicator for physicians to detect BTT. No single red cell index or formula offers complete sensitivity, specificity, and efficacy (100.0%) in distinguishing beta thalassemia traits from other forms of hypochromic microcytic anemia. The diversity of thalassemia mutations significantly influences these variations.

LIMITATIONS

The results of this study are biased as only selected samples were tested using conventional methods. Moreover, the high incidence of iron deficiency anemia in the population and the study cohort is another limiting factor that could be a reason for the underdiagnosis of beta thalassemia trait.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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2	**Saba Aman:** Data collection.
3	**Muhammad Younus Jamal Siddiqi:** Drafting, critical review.
4	**Shazia Kousar:** Data collection.
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