Open Access

Original Article

Differentiation of Beta-Thalassemia Trait (β-TT) from Iron Deficiency Anaemia (IDA) by Haematological Indices

Mehir Jabeen, Hamid Iqbal, Azeema Ahmed, Javaria Ahsan, Umer Shujaat, Nadia Tayab

Department of Pathology, Combined Military Hospital, Quetta/National University of Medical Sciences (NUMS) Pakistan

ABSTRACT

Objective: to analyze and compare the accuracy of six haematological indices based on red blood cell (RBC) parameters in distinguishing between beta-thalassemia and iron deficiency anaemia.

Study Design: Cross-sectional study.

Place and Duration of Study: Pathology Department, Combined Military Hospital, Quetta Pakistan, from Feb 2020 to Nov 2021.

Methodology: Ninety study samples were included and referred to the Pathology department for the hypochromic microcytic blood picture workup. All the samples were subjected to Complete Blood Counts and haemoglobin electrophoresis. A test for serum ferritin level was also carried out on all individuals. Blood morphology, Mean Corpuscular Volume (MCV), Red Cell Distribution Width (RDW), and Red Blood Cell (RBC count) were noted from an automated haematology analyzer (SYSMEX X100). Six haematological discrimination indices for diagnostic performance were tested, namely: Mentzer index, Srivastava index (SI), Red Cell Distribution Width Index(RDWI), England and Fraser index(EF), Green and King (GK), and Shine and Lal index (SL), to analyze the differences between β-TT and IDA by using evaluation calculated from RBC indices by various mathematical formulae.

Result: For delineating of*β*-TT from IDA, the Mentzer index is simple to use and accurate. In our study, the highest percentage of patients correctly diagnosed, 261(90%), was by the Mentzer index; the second-highest was by the RDWI 247(85%), and the third was Srivastava Index.

Conclusion: In this study, the Mentzer index was a reliable and helpful index for differentiation between *β*-TT and IDA, as compared to another index.

Keywords: Beta-thalassemia trait, Iron deficiency anemia, Red blood cell indices.

How to Cite This Article: *Jabeen M, Iqbal H, Ahmed A, Ahsan J, Shujaat U, Layab N. Differentiation of Beta-Thalassemia Trait (β-TT) from Iron Deficiency Anaemia (IDA) by Haematological Indices. Pak Armed Forces Med J 2023; 73(3): 829-832. DOI: https://doi.org/10.51253/pafmj.v73i3.8601*

INTRODUCTION

Thalassemia is a group of hereditary blood disorders that affects people worldwide, and it is estimated that 3% of the population is affected by beta-Thalassemia. Every year 60,000 children are born with Thalassemia in which; 80% of these children are born in Asian countries.[1] Beta Thalassemia is usually prevalent in the Eastern and Southwestern provinces of Saudi Arabia, where consanguine marriages are more than 50%.[2] Thalassemia is identified in 5000/year in Pakistan. In our country, the carrier rate is around 5-8%.[3] There is currently no national screening scheme set to detect the status of the beta-thalassemia trait in Quetta, Baluchistan. Nevertheless, a small study showed it to be 6.5% in 2012.[4,5] The outcome of Hemoglobin electrophoresis and serum ferritin estimation is often used to distinguish between β-TT and IDA. *β*-TT individuals are generally asymptomatic and may remain unaware of their carrier status until diagnosed by testing. Therefore, Thalassemia is the most common hereditarily transmitted hemoglobinopathy.[6]

The other necessity to correctly diagnose β-TT and IDA is that all those patients of β-TT having a wrong diagnosis of IDA if they get married to β-T carrier can have offspring suffering from beta thalassemia major-1.[7] To determine red cell indices that may indicate *β*-TT, Red blood cell counts and related indices obtained with automated haematology analyzers have been employed for a long. Since 1970 several Complete Blood Count (CBC) indices have been presented as an inexpensive and straightforward method for detecting if a blood sample is indicative of *β*TT or IDA1.[8,9] The potential of using RBC indices to discriminate IDA and-β-TT lies in detecting subjects with a high possibility, who may require suitable follow-up and decreased unnecessary investigative costs.[10]

The objective of the study is to determine the clinical significance and effectiveness, of specific haematological indices, in distinguishing beta-thalassemia (β-TT) from iron deficiency anaemia (IDA)

Correspondence: Dr Mehir Jabeen, Department of Haematology, Combined Military Hospital, Quetta, Pakistan

Received: 20 Apr 2022; revision received: 28 Oct 2022; accepted: 03 Nov 2022

in the population of Quetta. Consequently, highlighting the indicators is best for reducing unnecessary investi-gational costs put on the health care system.

METHODOLOGY

The cross-sectional study was conducted at Pathology Department, Combined Military Hospital, Quetta Pakistan, from February 2020 to November 2021. The current study was approved by the CMH Quetta Institute Review Board (ERC no. CMHQTA-IRB/039).

Inclusion Criteria: Patients of either gender or age groups having hypochromic, microcytic blood indices were included in the study.

Exclusion Criteria: Patients with diagnosed malig-nancies and inflammatory/infectious diseases were excluded from the study based on clinical data and personal information obtained from medical records. In addition, pregnant females with severe physical complaints and patients with a blood transfusion his-tory in the preceding four weeks were also excluded.

Non-probability Consecutive sampling was done, and regardless of age, gender and ethnicity, study participants were selected. A proforma was composed to include demographic data of patients like age, gender, ethnicity, clinical signs and symptoms, and past medical and surgical history.

Two samples of venous blood, 2ml each, were collected in EDTA and plain tube from every indiv-idual using an aseptic technique. On the same day, CBC parameters were calculated using an automated haematology analyzer (SYSMEX X100). All samples were subjected to peripheral blood morphology, and serum ferritin levels were calculated. Haemoglobin (Hb) electrophoresis was performed on cellulose acetate for all the participants. All participants were classified as IDA or β-TT based on their Serum Ferritin level and Hb electrophoresis findings.

Patients with serum ferritin levels <12ng/ml were identified as IDA cases, while with HbA2, more than 3.2% were identified as β-TT cases 2.[11] The discrimi-nation factors between β-TT and IDA were computed, confirmed, and correlated(Table-I).

Specificity, sensitivity, positive predictive value (PPV), negative predictive value (NPV), and Youden's indexes were calculated for each index. Data were analyzed using Statistical Package for the social sciences (SPSS) version 19.00 and MS Excel 2016 software. The Median and IQR were calculated for the continuous variable. Frequency and percentage were calculated for categorical variables. Mann-Whitney U test was used to differentiate IDA, and the *p*-value of ≤0.05 was considered significant.

Table-I: Difference β-TT and IDA, Various RBC Indices Based Mathematical Formulae

Hematological indices	Formulae	β-TT Cut-Off Value	IDA Cut-Off Value
Mentzer index (1973)	MCV/RBC	<13	>13
Shine and Lal (1977)	MCVxMCVx MCH/0.01	<1530	>1530
England and Fraser (1973)	MCV-(5xHb)-RBC-5.19	<0	>0
Srivastava (1973)	MCH/RBC	<3.8	>3.8
Green and King (1979)	MCVxMCVxRDW/Hbx100	<65	>65
Red Cell Distribution Width Index	MCVxRDW/RBC count	<220	>220

RESULT

Of 290 samples, 110(37.9%) were males, and 180(62.1%) were females. The age of study participants ranged between 9-50 years (mean age 20.10±11.25). A total of 290 patients were included in the study; 290,135 were identified with Iron deficiency anaemia, and 155 revealed an HbA2 value of >3.5%, recognized as diagnostic for the Beta Thalassemia trait. Their ages range from 9-50 years (mean±SD 20.1069±11.255). Table-II shows the Red blood cell parameters of both the groups of IDA and β-TT. As shown in Table-III, none of the indices analyzed showed 100% accuracy in distinguishing *β*-TT. Consequently, our study showed that this particular index could not be used for screening of B-TT, as using it can lead to a large number of false-negative cases reported. The index with the lowest sensitivity of 60.2% was England and Fraser (EF) index. On the other hand, the highest specificities for B-TT were found with the Mentzer index and England and Fraser index, with 82.3% and 85%, respectively. Table-III also indicates that the Mentzer index was the highest (84%), and the Shine and Lal had the lowest PPVs (52%).

Regarding the accuracy of the indexes to differentiate *β*-TT and IDA, Youden's index revealed the following ranking: (Mentzer > RDWI > Srivastava > Green and King > England and Fraser > Shine and Lal). Youden's indices of Mentzer index and shine and Lal were the highest and lowest, (85 and 11.8)

respectively. None of the indexes was sensitive or specific when discriminating between β-TT and IDA.

parameter. In 61% of cases with mild anaemia, one past found an elevated RBC count.[12]

Table-II: Red Cell parameters in Beta-Thalassemia Trait and Iron Deficiency Anemia in subjects with hypochromic microcytic blood picture

CBC parameter	Iron Deficiency Anemia		Beta-Thalassemia Trait		
	Range value	Median (IQR)	Range	Median (IQR)	*p*-value (t-test value)
Hb, (g/dl)	5.5-6.0	9(4.4,9,12.9)	9.0-15	9(4.4,9,12.9)	0.55
RBC(1012/l)	2.8-4.0	4(3.2,4,5.4)	4.9-6.4	4(3.2,4,5.4)	0.001
MCV(fl)	65.3-78.4	57.2(55,57,66.2)	52.1-57.7	57.2(55,57,66.2)	0.001
MCH	19.1-27.8	22(19,22,22.6)	15.7-25.5	22(19,22,22.6)	0.001
MCHC	24.8-38.7	29(27.3,29.6,31)	27.3-40.9	29(27.3,29.6,31)	0.001
Ferritin(µg/l)	2.1-9.6	10(6.8,10.5,53)	10.5-87.4	10(6.8,10.5,53)	0.001
RDW	12.4-13.8	13(12,13,13)	11.6-18.8	13(12,13,13)	0.001

β-TT: Beta-Thalassemia Trait; IDA: Iron-Deficiency Anemia; Hb: Hemoglobin; RBC: Red Blood Cell; MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Hemoglobin: RDW: Red Blood Cell Distribution Width.

Table-III: Sensitivity, Specificity, Positive Predictive Value, Negative Predictive Value and Youden's Index of Six Hematological Indices were used to Distinguish, between Beta Thalassemia and Iron Deficiency Anemia (n=290)

	Sensitivity %	Specificity %	Positive Predictive Value %	Negative Predictive Value %	YI
Mentzer(MI)					
β-TT	96	82.3	84	98.7	85
IDA	80.3	96.7	98.7	84	
Shine and Lal					
β-TT	100	10.5	52	100	11.8
IDA	14.7	100	100	52	
Srivastava					
β-TT	84.7	72	75	80.2	56.2
IDA	72	84.7	80.6	75	
Green and King					
β-TT	83.1	73.5	77.6	79.3	56.6
IDA	73.5	83.1	79.3	77.6	
England and Fraser					
β-TT	60.2	85	83.6	71	44.2
IDA	85.7	65.5	66	83.6	
RDWI					
β-TT	82	75.4	80	78	60.5
IDA	76.4	83.1	80	80	

Y= Youden's index

DISCUSSION

In our study, except for Haemoglobin, the biochemical and haematological parameters of β-TT and IDA differed significantly; however, the index's reliability in the differential diagnosis of β -TT and IDA did not reflect similar changes. Out of 290 individuals with hypochromic microcytic blood pictures, 186 individuals (65.1%) had a high RBC greater than $5.0\times10^6/\mu L$ at the time of diagnosis. 30.4% of individuals with IDA, on the other hand, had a high RBC count. Therefore it was concluded that to distinguish β-TT from IDA, RBC count alone was not a reliable

Youden's index evaluates both specificity and sensitivity to produce a valid evaluation for a specific technique. Another study showed, for correctly differentiating β-TT and IDA, England & Fraser (EF) index had the greatest Youden index score of 98 % for accurately distinguishing IDA from β-TT, while the Shine and Lal failed to distinguish microcytic anaemia.[13] In our study, for correctly diagnosing-TT and IDA, Mentzer Index (MI) had the greatest Youden index of 85%. England and Fraser and the Shine and Lal indices values had low Youden's index values of 44% and 11.8%, respectively. In a study conducted in Pakistan in 2019, the sensitivity of the seven discriminating indexes was calculated. Mentzer Index showed the highest sensitivity (92.56%), followed by RDWI (91.70%) and Srivastava (79%).[1] According to our findings, the Shine and Lal had the highest sensitivity (100%), followed by the Mentzer index (96%)and the Srivastava index; sensitivity was 83.1%.

Another study concluded that among people with mild to moderate microcytic anaemia aged 1.8–7.5 years, the RDWI and Green and King had the most sensitivity (78%) and specificity (80%).[14] In contrast, England and Fraser had high specificity (99.1%), while Youden had a high specificity (64.2%). One study claimed that in the beta thalassemia trait, patients' sensitivity was highest for Shine and Lal index (95%), followed by the Mentzer index (52% sensitivity), and then the Srivastava index (46% sensitivity). The red cell distribution width index had poor sensitivity of 2%.[15] Another study observed that in 153 patients with β-TT and 170 with IDA, Youden's index of beta thalassemia show that under the age of 10 years, Shine and Lal index and Red Blood Cell has the highest value for diagnostic purpose 89% and 82%, respectively.[16] A previous study concluded that Mentzer index showed

85% sensitivity, 93% specificity 19. Mentzer, Srivastava, RDWI, and MCHD sensitivities were 92.56%, 91.70%, 79%, and 70.9%, respectively.[17] According to our result, the most sensitive index for detecting beta Thalassemia trait is Shine and Lal.

The HbA2 percentage of RBCs is measured using Hemoglobin electrophoresis or HPLC to diagnose β-TT. Because studies have demonstrated that iron deficiency directly impacts HbA2 synthesis rates in the bone marrow, 16–20 weeks of iron therapy should be initiated, followed by a repeat serum iron with electrophoresis to ensure improvement in HbA2 levels.[18]

ACKNOWLEDGEMENT

We thank the Combined Military Hospital Quetta, Blood Banks, Pathology laboratory and volunteers for participating in this study.

CONCLUSION

Haematological indices, especially the Mentzer index, are a reliable and helpful method for the initial screening of microcytic hypochromic anaemia and are better than other methods used in this study in differentiating β -TT from iron deficiency anaemia.

Conflict of Interest: None.

Authors' Contribution

Following authors have made substantial contributions to the manuscript as under:

MJ & HI: Conception, data analysis, drafting the manuscript, approval of the final version to be published.

AA & JA: Data acquisition, drafting the manuscript, critical review, approval of the final version to be published.

US & NL: Study design, drafting the manuscript, data interpretation, critical review, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

REFERENCES

1. Mustafa A, Ali BA, Zulfiqar M, Naseem L. Role of discrimination indices in screening of beta thalassemia trait in low-resourced areas of Pakistan. Nat J Health Sci 2020; 4(1): 21-24.
2. Jameel T, Baig M, Ahmed I, Hussain MB, Alkhamaly MB. Differentiation of beta thalassemia trait from iron deficiency anemia by hematological indices. Pak J Med Sci 2017; 33(3): 665-669. https://doi: 10.12669/pjms.333.12098.
3. Aziz M, Anwar MJ. Prevalence of beta-thalassemia trait in quetta city, cross section study. J Univ Med Dent Coll 2015; 6(4): 21-26.
4. Roth IL, Lachover B, Koren G. Detection of β-thalassemia carriers by red cell parameters obtained from automatic counters using mathematical formulas. Mediterr J Hematol Infect Dis 2018; 10(1): e2018008. https://doi: 10.4084/MJHID.2018.008.
5. Urrechaga E, Hoffmann J. Critical appraisal of discriminant formulas for distinguishing thalassemia from iron deficiency in patients with microcytic anemia. Clin Chem Lab Med 2017; 55(10): 1582-1591. https://doi: 10.1515/cclm-2016-0856.
6. Tripathi N, Soni JP, Sharma PK, Verma M. Role of haemogram parameters and RBC indices in screening and diagnosis of beta-thalassemia trait in microcytic, hypochromic Indian children. Int J Hematol Dis 2015; 2(2): 43-46. https://doi: 10.12691/ijhd-2-2-4
7. Jahangiri M, Rahim F. Application of bayesian decision tree in hematology research: Differential diagnosis of β-thalassemia trait from iron deficiency anemia. Comput Math Method Med 2021; 2021: 6401105. https://doi: 10.1155/2021/6401105.
8. Mentzer WC Jr. Differentiation of iron deficiency from thalassaemia trait. Lancet 1973; 1(7808): 882. doi: 10.1016/s0140-6736(73)91446-3.
9. Shine I, Lal S. A strategy to detect beta-thalassaemia minor. Lancet 1977; 1(8013): 692-694. https://doi: 10.1016/s0140-6736(77)92128-6.
10. 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): 481-491.
11. Ehsani MA, Shahgholi E, Rahiminejad MS, Seighali F, Rashidi A. A new index for discrimination between iron deficiency anemia and beta-thalassemia minor: results in 284 patients. Pak J Biol Sci 2009; 12(5): 473-475. https://doi: 10.3923/pjbs.2009.473.475.
12. Klee GG, Fairbanks VF, Pierre RV, O'Sullivan MB. Routine erythrocyte measurements in diagnosis of iron-deficiency anemia and thalassemia minor. Am J Clin Pathol 1976; 66(5): 870-877. https://doi: 10.1093/ajcp/66.5.870.
13. England JM, Fraser PM. Differentiation of iron deficiency from thalassaemia trait by routine blood-count. Lancet 1973; 1(7801): 449-452. doi: 10.1016/s0140-6736(73)91878-3.
14. Srivastava PC. Differentiation of thalassaemia minor from iron deficiency. Lancet 1973; 2(7821): 154-155. https://doi: 10.1016/s0140-6736(73)93104-8.
15. Telmissani OA, khalil S, Roberts G. Mean density of hemoglobin per liter of blood: a new hematologic parameter with an inherent discriminant function. Lab Haematol 1999; 5(1): 149-152.
16. Aslan D, Altay C. Incidence of high erythrocyte count in infants and young children with iron deficiency anemia: re-evaluation of an old parameter. J Pediatr Hematol Oncol 2003; 25(4): 303-306. https://doi: 10.1097/00043426-200304000-00007.
17. Vehapoglu A, Ozgurhan G, Demir AD, Uzuner S, Nursoy MA, Turkmen S, *et al*. Hematological indices for differential diagnosis of Beta thalassemia trait and iron deficiency anemia. Anemia 2014; 2014: 576738. https://doi: 10.1155/2014/576738.
18. Ravanbakhsh M, Mousavi S. Diagnostic reliability check of red cell indices in differentiating iron deficiency anemia (IDA) from beta thalassemia minor (BTT). Hormozgan Med J 2016; 20(3): 151-157.