ORIGINAL ARTICLE

# Haematological indices as screening tool in distinguishing beta thalassemia trait and iron deficiency anaemia: Insights from a tertiary hospital study

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**ABSTRACT**

**Introduction: Beta thalassemia trait (BTT) or heterozygous, is an inherited haematological disease characterised by more than 100 different mutations that lead to reduced beta chain formation. Distinguishing BTT from Iron deficiency anaemia (IDA) is challenging since both conditions are characterised by microcytic anaemia, have distinct underlying causes, and thus require different approaches in treating the condition. The aim of our study is to determine the utility of haematological indices as a screening marker in detecting cases of beta thalassemia carriers and differentiating it from IDA in our tertiary care hospital.**

**Materials and Methods: A retrospective analysis of the data at the Department of Pathology from January 2020 to December 2023 (4 years duration) was conducted. The analysis included a comparison of haematological parameters and RBC (Red Blood Cell) indices between the 78 cases of BTT and the 60 cases of IDA. Complete blood count (CBC) samples were collected in 2 ml of K2 EDTA (Di potassium Ethylene Diamine Tetra Acetic acid) vacutainer tube and processed in the automated haematology analyser, Sysmex XN 1000. Haematological parameters were analysed, and RBC indices were calculated. HPLC (High Performance Liquid Chromatography) analysis was done to detect HbA2/F levels. HbA2 levels more than 4% were diagnosed as BTT in our study. Various discriminating formulas, such as Srivastav index(SI), Red blood cell Distribution Width Index (RDWI), Shine and Lal index (SLI), Ricera index (RI), Green and King index (GKI), Mentzer index (MI), Sirdah Index (SI), Mean density of Haemoglobin/litre of blood (MDHL), England and Fraser index (EFI), Mean Cell Haemoglobin Density (MCHD), and Ehsani Index (EI), were applied to differentiate BTT cases from IDA Cases.**

**Results: The mean haemoglobin in BTT was 10.8 g/dl, while in IDA, the mean Hb was 7.4 g/dl. The mean RBC count in BTT was 5.6 million/mm3, while in IDA it was 3.8 million/mm3. The values of haemoglobin (HB), Red blood cell Distribution Width – Coefficient of Variation (RDW-CV), Mean Corpuscular Haemoglobin Concentration (MCHC) and Mean Corpuscular Volume (MCV), between BTT cases and IDA cases showed statistical significance of $p<0.05$. Among the 11 discriminator formulas applied in our study, maximum accuracy was observed with the Ehsani index (84.06%), followed by the MI (82.61%). Maximum sensitivity was observed with RDWI (97.44%), followed by SLI (96.15%), EI (83.3%), and MI (79.45%). Youdens' score was highest in Ehsani index (68.33%), followed by MI (66.16 %), and the Green and King index (59.87%).**

**Discussion: Among the various discriminator formulas applied, two indices, such as EI and MI showed the highest accuracy, and maximum score in Youdens' formula. Hence, in mass screening or in cases suspected to have IDA, either MI <13 or Ehsani index <17 with normal serum ferritin levels can be subjected to HPLC evaluation for the diagnosis of beta thalassemia carrier cases.**

**KEYWORDS:**
*BTT, Mentzer index, Microcytic hypochromic anaemia, High performance liquid chromatography*

## INTRODUCTION

Beta thalassemia trait (BTT), or heterozygous, is an inherited haematological disease characterised by more than 100 different mutations that leads to reduced beta chain.[1] BTT is indeed often clinically asymptomatic or presents with minimal symptoms.[2] Distinguishing between BTT and Iron deficiency anaemia (IDA) are challenging, since both conditions are characterised by microcytic anaemia, have distinct underlying causes and thus require different approaches in treating the condition.[3] BTT generally doesn't require iron supplementation unless there is a co-existing iron deficiency.[4] Unnecessary iron supplementation in individuals with BTT can lead to iron overload.[5] BTT carries groups, implications, if both the parents are carriers of the BTT.[6] Several formulas and Red blood cell indices (RBCI) are formulated to differentiate between IDA and BTT.[7] RBC parameters such as mean corpuscular volume(MCV) and mean corpuscular haemoglobin concentration (MCH) are lower in cases of BTT as well as in IDA while the RBC count is often normal or even elevated in the cases of BTT and lower in IDA.[8,9] discriminating formulas such as Srivastav index(SI),Red cell distribution width index (RDWI), Mentzer index (MI), Ricera index( RI), Shine and Lal index(SLI), Green and King index(GKI), Sirdah Index( SI), Mean density of Haemoglobin/litre of blood (MDHL), England and Fraser index(EFI), Mean Cell Haemoglobin Density(MCHD) and Ehsani Index (EI ) are nowadays utilised as a screening method in distinguishing BTT cases from IDA.[10-13] Hence proper differentiation of BTT with RBC indices and Discriminating formula allows appropriate genetic

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counselling for affected individuals and their families, informing them about the risks and implications for their children. The aim of our study is to determine the utility of haematological indices, as a screening marker in detecting cases of Beta thalassemia carrier and in differentiating it from IDA in our tertiary care hospital.

## MATERIALS AND METHODS

*Study design:*
A retrospective study was conducted in our institution, in the Department of Pathology, for a duration of four years (January 2020- December 2023). This study received clearance from the institutional ethics committee with an IEC Reference number 008/12/2022/IEC/SMCH.

*Inclusion criteria:*
The study included a comparison of haematological parameters and RBC indices between the two groups BTT (78 cases) and IDA (60 cases). Cases with HbA2 more than 4% in HPLC study were considered BTT. Cases of IDA were diagnosed based on the iron studies that were included for comparison.

*Exclusion criteria:*
Cases with a history of blood transfusion less than three months and other haemoglobinopathies were excluded from the study.

*Study methodology:*
Complete blood count was analysed using Sysmex automated haematology analyser XN1000. Haematological parameters such as HB, RDW, Haematocrit, RBC, MCV, MCHC and Mean corpuscular haemoglobin (MCH) were observed. The following Indices as SRI, RDWI, MI, RI, SLI, GKI, SI, MDHL, EFI, MCHD and EI were calculated and statistically analysed using SPSS statistical package version 23 and JASP version 0.18.3. Chi-square test and Anova test were used to compare between the two groups BTT with IDA. A $p<0.05$ was generally considered to be statistically significant. Receiver operating characteristic (ROC) curve analysis was completed using the same software to elicit the area under the curve (AUC) for the red cell indices and discriminator formula. The point of the curve where sensitivity and specificity were almost equal was considered as the optimal cut off for the red cell parameters.

## RESULTS

*Age and gender distribution among BTT and IDA cases:*
This study included 78 cases of BTT and 60 cases of IDA. The age group included among BTT cases were between 1 year and 56 years, and the age group of the IDA cases were between 1 year - 49 years. The mean age in BTT was 26 years and the mean age in IDA was 24 years. The male to female ratio in BTT was 1.29:1 and in IDA was 7.5:1. Majority of IDA cases were found to be females, whereas most of BTT cases were found to be males.

*Analysis of haematological parameters in BTT and IDA cases:*
The mean haemoglobin in BTT includes 10.8 g/dl with a range of 4.8g/dl to 17 g/dl. While in IDA mean Hb was 7.4 g/dl with a range of Hb 3.6 g/dl to 10.3 g/dl. Mean RBC count in BTT was 5.6 million/mm3 while in IDA was 3.8 million/mm3. Mean MCV, MCH, MCHC and RDW-CV values in BTT were 63.5 fl, 19.6 pg, 30.7g/dl and 18.2% retrospectively. While in IDA, mean values of MCV, MCH, MCHC and were as 69.6 fl, 19.3 pg, 27.6g/dl and 19.9% retrospectively. The values of HB, MCV, MCHC and RDW-CV between BTT and IDA show statistical significance with p value <0.05, tabulated in Table I.

*Various formulas used to differentiate BTT and IDA cases:*
Table II showed the various discriminating indices in differentiating BTT from IDA. Statistical significance of $p<0.05$ was seen in indices such as MI, EFI, GKI, SRI, RI, SI, EI, RDWI and MDHL.

*Statistical analysis of various index in BTT cases:*
In this study accuracy, sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and Youden's index were calculated for the following haematological indices such as MI, GKI, SRI, RIC, MCHD, RDWI, SLI, EFI, SI, EI and MDHL

The formula used for accuracy, sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and Youden's index are as follows:
Accuracy = (True positive +True negative) (True positive +True negative +False positive +False negative)] × 100

sensitivity=True positive /True positive + False negative × 100

specificity=True negative/True negative + False positive × 100
positive predictive value (PPV)=True positive /True positive + False positive × 100

negative predictive value (NPV) =True negative/True negative + False negative × 100
Youden’s formula = (Sensitivity + Specificity) – 100

The maximum accuracy was observed in, EI (84.06%) followed by the MI (82.61%). The maximum sensitivity was observed in RDWI (97.44%), SLI (96.15%), EI (83.3%) and MI (79.45%) retrospectively. Youdens' score was best in EI 68.33%, followed by MI 66.16 % and GKI 59.87% retrospectively.

*Receiver operating characteristic (ROC) curve for Mentzer index:*
The diagnostic efficacy of MI for predicting thalassemia cases was assessed using the ROC curve as shown in figure 1. ROC plots displayed sensitivity versus 1-specificity, such that area under the curve generated was 0.89, indicating increased discriminating ability.

## DISCUSSION

The most common cause of anaemia in developing countries is IDA. Microcytic picture is seen in both cases of IDA and BTT cases hence diagnosis of BTT is often missed, all microcytic anaemia is managed with iron treatment. There is a chance of 25 % risk of having thalassemia major baby in each pregnancy if both the parents are of beta thalassemia carrier. Many formulas and indices are used as a screening tool to

**Table I: Characteristics of haematological parameters in BTT vs. IDA**

| Parameters | MCV (fl) | MCH (pg) | MCHC (g/dl) | RDW-CV (%) | HB g% | RBC in millions |
|---|---|---|---|---|---|---|
| | | | BTT | | | |
| Mean | 63.5 | 19.6 | 30.7 | 18.2 | 10.8 | 5.6 |
| SD | 7.2 | 2.6 | 1.6 | 3.0 | 2.2 | 1.1 |
| Minimum | 52.4 | 14.6 | 25.6 | 13.5 | 4.8 | 1.49 |
| Maximum | 98 | 32.2 | 34 | 30.2 | 17 | 7.69 |
| | | | IDA | | | |
| Mean | 69.6 | 19.3 | 27.6 | 19.9 | 7.4 | 3.8 |
| SD (Standard Deviation) | 9.9 | 4.6 | 2.9 | 4.1 | 1.7 | 0.63 |
| Minimum | 53 | 9.2 | 21.1 | 13.2 | 3.6 | 2.6 |
| Maximum | 90 | 29.4 | 33.1 | 30.0 | 10.3 | 5.3 |
| p-value Significance between BTT & IDA group | <0.001 | 0.646 | <0.001 | 0.006 | <0.001 | <0.001 |

**Table II: Discriminating formula in differentiating cases of BTT vs. IDA**

| INDEX | FORMULA | CUT OFF | NO OF CASES | BTT | IDA | p value |
|---|---|---|---|---|---|---|
| MI | MCV | 13 | < 13 | 62 | 8 | <0.001* |
| | RBC | | >13 | 16 | 52 | |
| GKI | (MCV × MCV × RDW) | 65 | <65 | 48 | 1 | <0.001* |
| | (Hb × 100) | | >65 | 30 | 59 | |
| EFI | MCV – (5 × Hb) – RBC – 3.4 | 0 | <0 | 45 | 0 | <0.001* |
| | | | >0 | 33 | 60 | |
| MCHD | MCH/MCV | 0.35 | >0.35 | 0 | 1 | 0.33 |
| | | | <0.35 | 78 | 59 | |
| SRI | MCH | 3.8 | <3.8 | 57 | 13 | 0.001* |
| | RBC | | >3.8 | 21 | 47 | |
| RI | RDW | 4.4 | >4.4 | 7 | 45 | <0.001* |
| | RBC | | <4.4 | 71 | 15 | |
| SI | MCV – RBC – (3 × Hb) | 27 | 27 | 50 | 0 | <0.001* |
| | | | >27 | 28 | 60 | |
| EI | MCV – (10 × RBC) | 17 | <17 | 65 | 9 | <0.001* |
| | | | >17 | 13 | 51 | |
| MDHL | (MCH/MCV) × RBC | 1.67 | >1.67 | 45 | 0 | <0.001* |
| | | | <1.67 | 33 | 60 | |
| SLI | (MCV )2× MCH/100 | 1530 | <1530 | 75 | 50 | 0.190 |
| | | | >1530 | 3 | 10 | |
| RDWI | MCV × RDW/RBC | 220 | <220 | 76 | 60 | 0.0318* |
| | | | >220 | 2 | 0 | |
| RBC in million/$mm^3$ | | 5.5 | >5.5 | 45 | 0 | <0.001* |
| | | | <5.5 | 33 | 68 | |

*Statistically significant MI- Mentzer index, RDWI- Red blood cell Distribution Width Index, SLI- Shine and Lal index, RI- Ricera index, GKI- Green and King index, SI- Sirdah Index, MDHL- Mean density of Haemoglobin/litre of blood, EFI- England and Fraser index, MCHD- Mean Cell Haemoglobin Density, EI- Eshani Index.

**Table III: Accuracy /Sensitivity /specificity /PPV/NPV/Youdens' indices in BTT cases**

| INDEX | Sensitivity (%) | Specificity (%) | Accuracy | PPV (%) | NPV (%) | Youdens' |
|---|---|---|---|---|---|---|
| MI | 79.49 | 86.67 | 82.61 | 85.57 | 76.47 | 66.16 |
| SLI | 96.15 | 16.67 | 61.59 | 60 | 76.92 | 12.82 |
| EFI | 57.69 | 100 | 76.09 | 100 | 64.52 | 57.69 |
| GKI | 61.54 | 98.33 | 77.54 | 97.96 | 66.29 | 59.87 |
| SRI | 73.08 | 78.3 | 75.36 | 81.43 | 69.12 | 51.41 |
| RI | 8.97 | 25 | 15.94 | 13.46 | 17.44 | 66.03 |
| SI | 64.1 | 100 | 79.71 | 100 | 68.18 | 64.1 |
| EI | 83.3 | 85 | 84.06 | 87.84 | 79.69 | 68.33 |
| MDHL | 57.69 | 100 | 76.09 | 100 | 64.52 | 57.69 |
| MCHD | 0 | 98.33 | 42.75 | 0 | 43.07 | -1.67 |
| RDWI | 97.44 | 0 | 55.07 | 55.88 | 0 | -2.56 |
| RBC | 57.69 | 100 | 76.09 | 100 | 64.52 | 57.69 |

MI- Mentzer index, RDWI- Red blood cell Distribution Width Index, SLI- Shine and Lal index, RI- Ricera index, GKI- Green and King index, SI- Sirdah Index, MDHL- Mean density of Haemoglobin/litre of blood, EFI- England and Fraser index, MCHD- Mean Cell Haemoglobin Density, EI- Ehsani Index.

**Table IV: comparison of sensitivity among the various discriminator formula between our study and other study**

| STUDY | Present study | Bhargava et al., study |
|---|---|---|
| RDWI | 97.4 | 70.4 |
| SLI | 96.1 | 100 |
| EI | 83.3 | 80.6 |
| MZ | 79.4 | 80.6 |
| SRI | 73 | 34.6 |
| SI | 64.1 | 77.5 |
| GKI | 61.5 | 78.5 |
| EFI | 57.6 | 77.5 |
| MDHL | 57.69 | 62.24 |
| RI | 8.9 | 100 |
| MCHD | 0 | 40.82 |
| RBC | 57.6 | 63.2 |

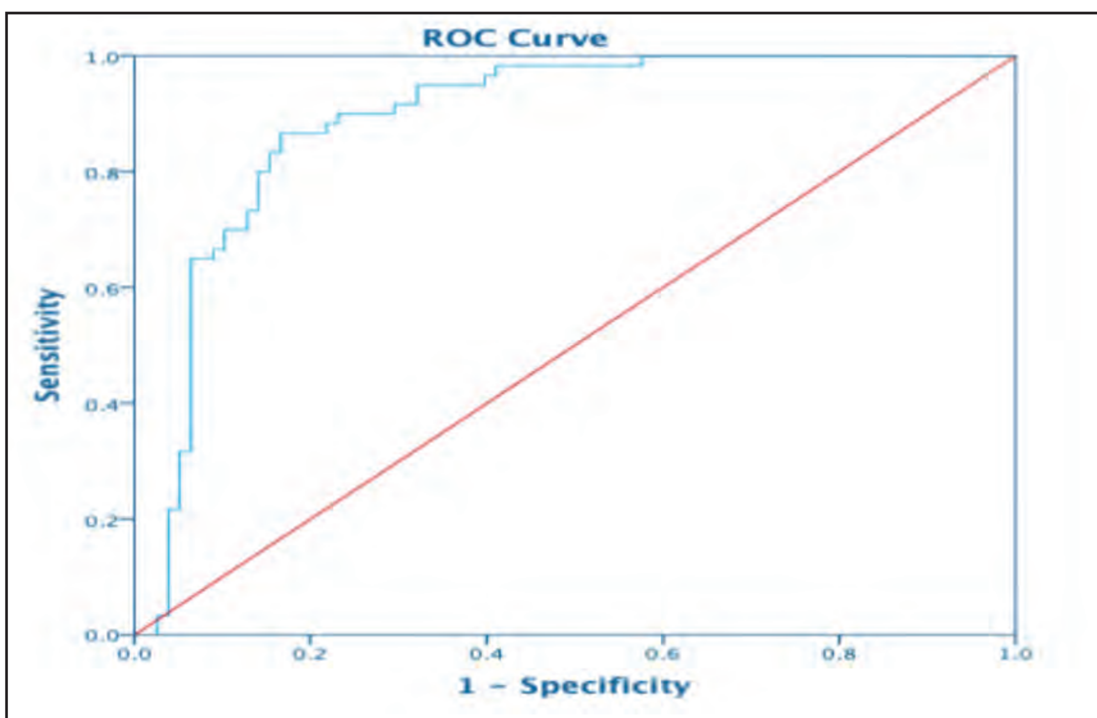


**Fig. 1:** ROC Curve - Mentzer index

differentiate BTT from IDA. These indices are helpful in mass screening. According to (TIF) the International Thalassemia Federation guidelines, MCH <27 pg, MCV <78 fl, and with microcytic, hypochromic picture findings in blood smear were suspected as carriers.[14] Our study showed that mean MCV as 63.5 fl and Mean MCH as 19.6pg. Mean RBC count in BTT is 5.6 million/cu.mm compared to the IDA of 3.8 millions.cu.mm which is the distinguishing finding in differentiating BTT. The values of HB, MCV, MCHC and RDW-CV compared between BTT and IDA show statistical significance of $p<0.05$ while the MCH does not show statistical significance.

The maximum sensitivity observed in our study was RDWI (97.44%) which was in concordance with the Jameel et al., study showed that 94% sensitivity for BTT cases[8] followed by RDWI index, SLI showed 96.15% sensitivity in present study similar to Bhargava M et al study showed 100% sensitivity. A study by Bordhar et al., showed 87.6% sensitivity for the cases of BTT.10,15 (Table IV)

In our research, Youden's highest score was observed in the EI at 68.33%, with the MI following closely behind at 66.16%, and the GKI at 59.87%. Conversely, in the study conducted by Bhargava et al, the highest Youden score was identified in the EFI at 89.92%, with the RIC index following closely behind at 87.39%.

In our research, the EI achieved the highest accuracy at 84%, succeeded by the MI at 82.6% and the SI index at 79.71%. Comparatively, when aligning our results with the Bhargava et al study, the GKI index exhibited the highest accuracy at 97.5%, with the RBC index closely trailing at 96.56%.

## CONCLUSION

The difference between IDA and BTT requires careful interpretation of red cell indices. Mentzer index is also helpful to distinguish IDA and BTT cases. In our study, among the various discriminator formulas applied, two indices, such as EI and MI, had shown highest accuracy and maximum score in the Youdens' formula. Hence in mass screening or in cases of suspected IDA, either MI <13 or EI <17 with normal serum ferritin levels should be subjected to HPLC study for the diagnosis of BTT cases. And also, antenatal women with microcytic hypochromic anaemia with normal iron profile should undergo HPLC screening.

## CONFLICT OF INTEREST

All co-authors had read and agreed the manuscript and there is no conflict of interest in this study.

## REFERENCES


1. Jaing TH, Chang TY, Chen SH, Lin CW, Wen YC, Chiu CC. Molecular genetics of β-thalassemia: A narrative review. Medicine (Baltimore) 2021; 100(45): e27522.
2. Origa R. β-Thalassemia. Genet Med 2017; 19(6): 609-19.
3. Kabootarizadeh L, Jamshidnezhad A, Koohmareh Z. Differential diagnosis of iron-deficiency anemia from β-thalassemia trait using an intelligent model in comparison with discriminant indexes. Acta Inform Med 2019; 27(2): 78-84.
4. Verma S, Gupta R, Kudesia M, Mathur A, Krishan G, Singh S. Coexisting iron deficiency anemia and Beta thalassemia trait: effect of iron therapy on red cell parameters and hemoglobin subtypes. ISRN Hematol 2014; 2014: 293216
5. Basu S, Rahaman M, Dolai TK, Shukla PC, Chakravorty N. Understanding the Intricacies of Iron Overload Associated with β-Thalassemia: A Comprehensive Review. Thalassemia Reports. 2023; 13(3): 179-94.
6. Noor FA, Sultana N, Bhuyan GS, Islam MT, Hossain M, Sarker SK, et al. Nationwide carrier detection and molecular characterization of β-thalassemia and hemoglobin E variants in Bangladeshi population. Orphanet J Rare Dis 2020; 15(1): 15.
7. Shuang X, Zhenming W, Zhu M, Si S, Zuo L. New logarithm-based discrimination formula for differentiating thalassemia trait from iron deficiency anemia in pregnancy. BMC Pregnancy Childbirth 2023; 23(1): 100.
8. 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): 665-9.
9. Wickramaratne KA, Wijewickrama DC. Screening for beta-thalassemia trait; applicability of red cell indices and parameters -A study in Sri Lanka. Int J Health Sci (Qassim) 2021; 15(1): 29-34.
10. Bhargava M, Kumar V, Pandey H, Singh V, Misra V, Gupta P. Role of hematological indices as a screening tool of beta thalassemia trait in Eastern Uttar Pradesh: an institutional study. Indian J Hematol and Blood Transfus 2020; 36(4): 719-24.
11. Kumar A, Saha D, Kini J, Murali N, Chakraborti S, Adiga D. The role of discriminant functions in screening beta thalassemia trait and iron deficiency anemia among laboratory samples. J Lab Physicians 2017; 9(03): 195-201.
12. Ebrahimpour Sadagheyani H, Sharafkhani R, Sakhaei S, Jafaralilou H, Shahmirzalou P. The evaluation of results of twenty common equations for differentiation of beta thalassemia trait from iron deficiency anemia: A cross-sectional study. Iran J Public Health 2022; 51(4): 929-38.
13. Jain AKK, Sharma P, Saleh S, Dolai TKK, Saha SCC, Bagga R, et al. Multi-criteria decision making to validate performance of RBC-based formulae to screen β-thalassemia trait in heterogeneous haemoglobinopathies. BMC Med Inform Decis Mak 2024; 24(1): 5.
14. Sari DP, Wahidiyat PA, Setianingsih I, Timan IS, Gatot D, Kekalih A. Hematological parameters in individuals with Beta thalassemia Trait in South Sumatra, Indonesia. Anemia 2022; 2022: 3572986.
15. Bordbar E, Taghipour M, Zucconi BE. Reliability of Different RBC indices and Formulas in Discriminating between β-thalassemia minor and other microcytic hypochromic cases. Mediterr J Hematol Infect Dis 2015; 7(1): e2015022.