Poorana Priya.P / Afr.J.Bio.Sc. 6(5) (2024). 9243-9253 **ISSN: 2663-2187**

https://doi.org/ 10.48047/AFJBS.6.5.2024. 9243-9253

African Journal of Biological Sciences

Utilization of Red cell indices and derived formulae in the diagnosis of Beta-Thalassemia trait among adult population in Chennai-A hospital based observational study.

Authors:

First author:

Poorana Priya.P,

Associate Professor,

Department of Pathology,

Saveetha Medical College and Hospital,

Saveetha Institute of Medical and Technical Sciences (SIMATS),

Saveetha University,

Thandalam,

Chennai.

Second Author:

Aravindan.K,

Post Graduate,

Department of Pathology,

Saveetha Medical College and Hospital,

Saveetha Institute of Medical and Technical Sciences (SIMATS),

Saveetha University,

Thandalam,

Chennai.

Article History
Volume 6, Issue 5, 2024
Received: 22 May 2024
Accepted: 03 Jun 2024
doi: ***10.48047/AFJBS.6.5.2024. 9243-9253***

ABSTRACT: Beta Thalassemia trait (BTT) is one of the heterozygousconditionsn where a single beta globin chain is affected, resulting in microcytic hypochromic anemia. [1,2,3] The estimated mean prevalence of BTT in India is around 3%. [4,5,6] Discrimination between iron deficiency anemia and BTT still remains as a diagnostic challenge, since both conditions share similar hematological abnormalities. [7,8,9] Definitive diagnosis and screening of BTT often help in establishing a suitable therapeutic strategy as well as in creating awareness among individuals. Hence in our study we have attempted to utilize the automated analyzer based red cell parameters and derived mathematical indices in detection of BTT. Our study results showed that there is a positive correlation between RBC count and age with HbA2 levels. .Hematological parameters such as MCV, RDW were found to be sensitive in distinguishing BTT from other microcytic anemias. Shine and Lal index showed the highest sensitivity of 98.7% and Srivatsava index had the maximum specificity of 96.1% in predicting BTT.

KEYWORDS: Beta thalassemia trait,Shine and Lal index, Srivatsava index,HbA2,

INTRODUCTION:

Beta thalassemia trait, a hereditary blood disorder caused by mutations in the beta-globin gene, leads to reduced hemoglobin production and subsequent microcytic hypochromic anemia. [10,11] Although often asymptomatic, BTT's clinical significance lies in its potential to be misdiagnosed as iron deficiency anemia or other forms of microcytic anemia, making accurate diagnosis crucial for appropriate management and genetic counseling. [12,13,14] The diagnosis of BTT typically involves hematological analysis, with red cell indices playing a vital role. Red cell indices provide critical insights into the morphology and size variation of erythrocytes. However, relying solely on these indices may not always suffice given the overlap with other microcytic anemias. This necessitates the utilization of derived hematological formulas that combine multiple red cell indices to enhance diagnostic accuracy. Several formulas have been developed to distinguish BTT from other microcytic anemias, including the Menzter index and Shine and Lal index. These

formulas leverage specific red cell parameters to create diagnostic thresholds that aid in the differentiation of BTT. This study aims to evaluate the effectiveness of red cell indices and derived formulas in diagnosing BTT in adult population .Based on this objectives of this study is to evaluate sensitivity and specificity of red cell parameters and derived indices for the differentiation of BTT from non-BTT groups and also to evaluate the association of HbA2 with specific hematological parameters (RBC,MCV, MCH and RDW) in Beta Thalassemia trait individuals.

METHODOLOGY:

A retrospective hospital based observational study was conducted for a total of 200 adult patients aged above 18 years who visited our hospital over a period of two years (March 2022-March 2024). The study was approved by institutional scientific review board , Approval number 141/04/2024/SRB/SMCH.

Patients with mild to moderate reduction in hemoglobin level corresponding to their age group were selected with a lower limit of 8.0 g/dl and with microcytic hypochromic anemia with MCV less than 80 fl were included in the study. Patients with Hemoglobin level less than 8.0 g/dl, Chronic disease or infection and history of previous blood transfusions were excluded

Sample collection and statistical analysis : For the cases meeting the above selection criteria the required data were collected from the medical records. 2ml of EDTA samples were collected by using a vacutainer system and a complete hemogram was done by using **SYSMEX XN-1000, 6 part** hematology analyzer. The 2-level controls were run daily and the instruments were maintained according to the instructions of the manufacturer. HbA2 estimation by High performance liquid chromatography was done using **BIO-RAD D-10** analyzer. Four mathematically derived red cell indices**,** Mentzer index, Shine and Lal index, Srivatsava index and Se**hg**al index were utilized in this study. Descriptive analysis and hypothesis testing were done by appropriate statistical tests by using commercially available statistical software packages.

RESULTS:

TABLE I:Baseline characteristics of our study:

Variables	BTT group		Non- BTT group	
	Mean	SD	Mean	SD

RBC[x10^{12}]	5.50	0.72	4.18	0.43
Hb[gm%]	10.57	1.60	9.41	0.70
Hct[%]	31.08	4.38	33.96	4.38
MCV[fl]	61.78	6.15	69.73	9.64
MCH[pg]	19.28	2.58	23.65	9.73
MCHC[gm%]	31.18	1.89	31.02	1.18
RDW-SD[%]	37.56	4.00	41.74	6.30
PLT[10^9/L]	288	79.31	319.92	111.73
HbA2(HPLC)	5.04	0.42	2.40	0.39
Mentzer index index	11.48	2.35	16.88	3.28
Srivastava index index	3.58	0.79	5.84	3.31
Shine and Lal index Lal	763.22	306.09	1132	318.27
Sehgal index index	721.15	234.41	1203.25	342.67

Table II depicts the original cut off value of the red cell indices using mathematical formulae as described by authors in various literatures among BTT and non BTT groups and are utilized in our present study for interpretation.

Table II: Cut off values of RBC indices utilized among BTT group:

Variables	BTT	Non-BTT
Mentzer index	<13	>13
Srivastava index	<3.8	>3.8
Shine and lal index	<1530	>1530
Sehgal index	<972	>972

The sensitivity and specificity of Mentzer index was 79.2% and 92.2 %, and that of Srivatsava Index was 71.6 % and 96.1% respectively, with a statistically significant p value of

<0.05. Similarly the sensitivity and specificity of Shine and Lal and Sehgal indices were 98.7% and 9.8%, 89.3 % and 78.4% respectively and also showed statistically significant p value of <0.05. We found highest sensitivity for Shine and Lal index and maximum specificity for Srivatsava index. (Table III)

TABLE III :Sensitivity,Specificity,PPV,NPV of various red cell indices utilized in our study:

Variables	Sensitivity [%]	Specificity [%]	PPV [%]	NPV [%]	Youden index[%]	Correctly identified cases	p-value
Mentzer index	79.2	92.2	96.7	60.3	71.4	75.33	<0.001
Srivastava index	71.6	96.1	98.1	53.8	67.7	71.33	<0.001
Shine and Lal	98.7	9.8	76.2	71.4	8.5	98.0	<0.001
Sehgal index	89.3	78.4	92.4	71.4	67.7	62.66	<0.001

PPV-Positive Predictive value,NPV-Negative Predictive value.

Table IV and Figure 1 depicts the Receiver Operating Characteristic (ROC) curves of different RBC indices along with Area under Curve of different indices used in our study. The Area Under Curve for Mentzer, Srivatsava and Sehgal indices were almost close to 1 with AUC of 0.994, 0.992, 0.936 and 0.881 for Shine and Lal index

Table IV: Receiver operative characteristic curves of different RBC indices along with Area under Curve of different indices used in our study:

Variables	Area under the curve	Standard error [a]	Asymptomatic Sig [b]	Asymptomatic 95% confidence interval	
				Lower bound	Upper bound
Mentzer index	0.994	0.003	0.000	0.988	1.000
Srivatsava index	0.936	0.016	0.000	0.904	0.968
Shine and Lal	0.881	0.026	0.000	0.830	0.932

index					
Sehgal index	0.936	0.004	0.000	0.0985	0.999

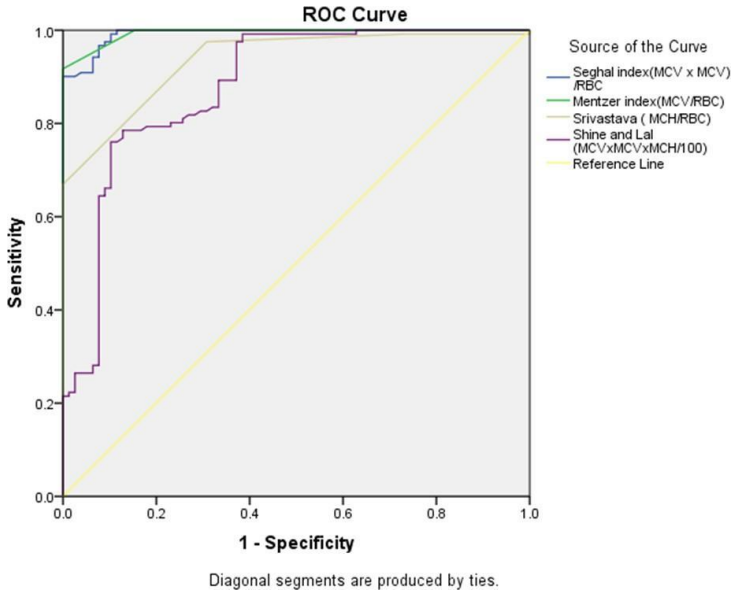

Figure 1 - ROC curves of the different indices under study

Table V and Figure 2 depicts the correlation of HbA2 with various variables like Age, RBC count, MCV, MCH and RDW and were found to be statistically significant with p-value of <0.05. We found that Age of the patient and RBC count had positive correlation with HbA2 with r value of 0.2668 and 0.5891 respectively and observed that variables MCV, MCH and RDW showing negative correlation with HbA2 with r value of -0.3886, -0.2716 and -0.3281respectively.

TABLE V:Pearson correlation with HbA2 and other parameters:

Variables	HPLC[HbA2%] [r value]	p-value
Age	0.2668	=0.0001
MCV	-0.3886	<0.0001
MCH	-0.2716	=0.0001
RDW	-0.3281	<0.0001
RBC	0.5891	<0.0001

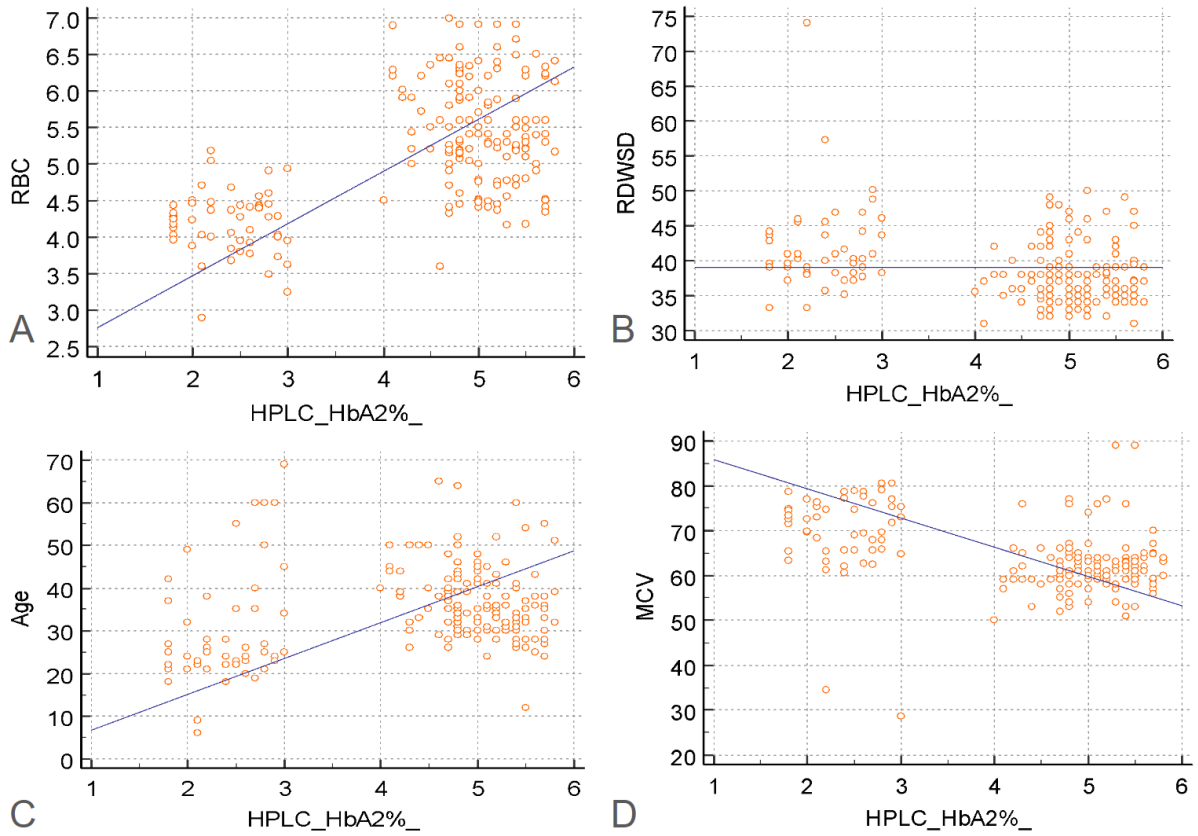

Figure 2 : A - Correlation between HbA2 and RBC. B - Correlation between HbA2 and RDW-SD. C - Correlation between HbA2 and Age. D - Correlation between HbA2 and MCV.

DISCUSSION:

The findings in our study highlight the diagnostic utility of red cell indices and derived formulae in distinguishing BTT from other forms of microcytic anemia. Our cohort consisted of 200 cases with a balanced gender distribution and significant number of BTT cases compared to non-BTT cases which is concordant with other studies. [15,16,17] The statistically significant differences observed in RBC count, MCV, MCHC, RDW and platelet values underscore the unique hematological profile associated with BTT and is similar to the studies done by Claude et al, Bhukhanvala D et al and Parthasarathy V et al. [18,19,20]

The mean RBC count was significantly higher in the BTT group compared to the non-BTT group and this aligns with the known compensatory mechanism in BTT where increased erythropoiesis maintains RBC numbers despite ineffective hemoglobin production. Similarly, the higher mean hemoglobin concentration in the BTT group versus the non-BTT group supports the presence of mild anemia typical of BTT rather than the more severe forms seen in other anemias. Several studies from Indian region have found the similar observations.[21,22,23]

Our data also revealed that MCV, MCH, MCHC values were lower in the BTT group which is consistent with microcytic hypochromic anemia characteristic of this condition. Conversely the RDW was higher in the non-BTT group reflecting greater variability in red cell size which is less typical in BTT. The elevated HbA2 levels in the BTT group is a definite marker which reinforces the diagnostic importance alongside traditional red cell indices. The ROC curves for Mentzer, Srivatsava and Sehgal indices demonstrated areas under the curve close to 1 with statistically significant sensitivity and specificity with p value <0.05 which indicates that the utility of combined indices can effectively distinguish BTT from other forms of microcytic anemia. But studies done by other authors had conflicting results. [24,25]

Shine and Lal index stands out with higher sensitivity and Srivatsava index with maximum specificity in diagnosing BTT, however these findings differed from other authors [26,27] and this could be region specificity. The study also explored correlations between patient demographics and hematological parameters with HbA2 levels and we found a positive correlation between patient age and RBC count with HbA2 which reflects the compensatory response to maintain adequate oxygen transport despite the reduced efficiency of hemoglobin in BTT. On the other hand MCV, MCH and RDW showed a negative correlation with HbA2 levels consistent with microcytic hypochromic nature of the red cells in BTT.

CONCLUSION:

Though HPLC analysis of hemoglobin remains the gold standard test for the diagnosis of Beta Thalassemia trait, red cell parameters and derived indices by automated counters can be utilized as a feasible tool in the detection of cases. These readily available parameters can be considered as an inexpensive method for mass screening of the population, which would enable the selection of samples for further analysis to confirm the diagnosis. We found that the RBC count, MCV, RDW and indices, particularly Shine and Lal and Srivatsava could be considered as sensitive markers in discriminating BTT from other microcytic anemias.

REFERENCES:

1. Kafle S and Lakhey M. Etiological study of microcytic hypochromic anemia. Journal of Pathology of Nepal. 2016, 6(12), pp.994-997.

2. Karimi M, Rasekhi A. Efficiency of premarital screening of beta-thalassemia trait using MCH rather than MCV in the population of Fars Province, Iran. Haematologia. 2002. 32(2):129-33. doi:10.1163/156855902320387961

3. Nigam N, Kushwaha R, Yadav G, Singh P, Gupta N, Singh B et al. A demographic prevalence of β Thalassemia carrier and other hemoglobinopathies in adolescent of Tharu population. Journal of Family Medicine and Primary Care. 2020;9(8):4305.

4. Balgir R. Prevalence of hemolytic anemia and hemoglobinopathies among the pregnant women attending a tertiary hospital in central India. Thalassemia Reports. 2015;5(1)..

5. Janel A, Roszyk L, Rapatel C, Mareynat G, Berger M, Serre-Sapin A. Proposal of a score combining red blood cell indices for early differentiation of beta-thalassemia minor from iron deficiency anemia. Hematology. 2011;16(2):123-127.

6. Aithal A V. Role of Red Cell Distribution Width Index for Differentiation of Iron Deficiency Anemia and Beta Thalassemia Trait. Indian Journal of Pathology: Research and Practice. 2020;9(2 (Part- I):117-120.

7.Madan N, Sharma S, Sood S.K, Colah R, et al. Frequency of beta thalassemia trait and other hemoglobinopathies in northern and western India. Indian Journal of human genetics, 2010; 16(1): 16-25.

8.Madan N, Sikka M, Sharma S, Rusia U, Kela K. Red cell indices and discriminant functions in the detection of beta thalassemia trait in a population with high prevalence of iron deficiency anaemia. Indian Journal of Pathology and Microbiology, 1999; 42(1): 55-61.

9. Rathod D, Kaur A, Patel V, Patel K, et al. Usefulness of cell counter-based parameters and formulas in detection of β -thalassemia trait in areas of high prevalence. Am J Clin Pathol., 2007; 128: 585-589.

10. Shinar E, Rachmilewitz EA. Oxidative denaturation of red blood cells in thalassemia. Semin Hematol., 1990; 27(1): 70-82.

11. Yuan J, Kannan R, Shinar E, et al. Isolation, characterization, and immunoprecipitation studies of immune complexes from membranes of beta thalassemic erythrocytes. Blood, 1992; 79(11): 3007-3013.

12. Shine I, Lal S. A strategy to detect β thalassaemia minor. Lancet, 1977; 1: 692-4.

13. Green R and King R. A new red cell desciminant incorporating volume dispersion for differentiating iron deficiency from thalassaemia minor. Blood Cells, 1989; 15: 481-95.

14. Jayabose S, Giavanelli J, LevendogluTugal O, Sandoval C, Ozkaynak F, Visintainer P. Differentiating iron deficiency anemia from thalassemia minor by using by an RDW-based index. J Pediatr Hematol.,1999; 21: 314.

15. Ehsani M.A., E. Shahgholi, M.S. Rahiminejad, F. Seighali, A. Rashidi. A new index for discrimination between Prachi Gupta, BP Nag, Abha Mathur. Evaluation of Red Cell Indices and Discriminant Functions in the Detection of Beta Thalassemia Trait. IAIM, 2019; 6(3): 50-59.

16. Ntaios G, Chatzinikolaou A, Saouli Z, Girtovitis F, Tsapanidou M, Kaiafa G, et al. Discrimination indices as screening test for beta thalassemia trait. Ann Hematol., 2007; 86: 487-91. 21.

17.Yasar M. Yousafzai, Shahtaj Khan, Fazle Raziq. β-thalassaemia trait: haematological parameters. J Ayub Med Coll Abbottabad, 2010; 22(4). 22.

18.Claude Owen Burdick. Separating Thalassemia Trait and Iron Deficiency by Simple Inspection. Am J Clin Pathol., 2009; 131: 444-445.

19. Bhukhanvala D, Seliya V, Shah A, Gupte S. Study of parents of βthalassemia major children to determine cutoff values of hematological parameters for diagnosis of βthalassemia trait and assessment of anemia in them. Indian J Med Sci., 2013; 67: 117–122. 24.

20.Parthasarathy V. Search for beta thalassemia trait in India. Turk J Hematol., 2012; 29: 427–429. 25.

21.Sehgal K, Mansukhani P, Dadu T, Irani M, Khodaiji S. Sehgal index: A new index and its comparison with other complete blood count-based indices for screening of beta thalassemia trait in a tertiary care hospital. Indian J Pathol Microbiol., 2015; 58(3): 310.

22. Matos JF, Dusse LM, Stubbert RV, Ferreira MR, Coura-Vital W, Fernandes AP, et al. Comparison of discriminative indices for iron deficiency anemia and β thalassemia trait in a Brazilian population. Hematology, 2013 May 1; 18(3): 169-74.

23. Okan V, Cigiloglu A, Cifci S, Yilmaz M, Pehlivan M. Red cell indices and functions differentiating patients with the β-thalassaemia trait from those with iron deficiency anaemia. J Int Med Res., 2009 Feb; 37(1): 25-30.

24. Nesa A, Tayyab A, et al. RDWI is better discriminant in differentiation of iron deficiency anaemia and Beta thalassemia trait. Bangladesh Journal of child Health, 2009; 33(3): 100-103.

25. Demir A, Yarali N, Fisgin T, Duru F, Kara AR. Most reliable indices in differentiation between thalassemia trait and iron deficiency anemia. Pedia Int., 2002; 44: 612-6.

26. Sirdah M, Tarazi E, Al-Najjar, AlHaddad R. Evaluation of the diagnostic reliability of

different RBC indices and formulas in the Differentiation of betathalassaemia minor from iron deficiency in Palestinian population. Int J Hematol., 2008; 30: 324-30.

27. Niazi M, Tahir M, e Raziq F, Hameed A. Usefulness of Red cell Indices in Differentiating Microcytic Hypochromic Anemias. Gomal J Med Sci., 2010 Dec 31; 8(2)