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Original Article

Pathology Section

Screening Beta Thalassemia Trait- Performance Evaluation of Discriminator Indices

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ABSTRACT

Introduction: Beta Thalassemia Trait (BTT) is the most common haemoglobinopathy worldwide. High Performance Liquid Chromatography (HPLC) is the technique of choice for diagnosis. However, it is expensive and there is a need to triage patients for HPLC in resource limited nations. Various discriminator indices are used for the same purpose.

Aim: To compare the utility of 11 different discriminator indices for differentiating between Iron Deficiency Anaemia (IDA) and BTT.

Materials and Methods: The present study was a retrospective observational study done at Maulana Azad Medical College, Delhi, India over a period of one year (January 2017-January 2018). A total of 510 cases with clinically suspected haemoglobinopathy or with microcytic hypochromic anaemia along with relative erythrocytosis were analysed. After noting clinical details, data regarding haemogram findings, iron parameters and serum vitamin B12 and folate levels were collected. An Haemoglobin A2 (HbA2) value of 3.8-8% was used for confirming diagnosis of BTT. Eleven discriminator indices prediciting BTT were calculated and their utility was assessed by calculating the sensitivity, specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV) and Youden's index. The statistical analysis was done using Statistical Package for the Social Sciences (SPSS) 16 software.

Results: Out of total 510 cases, 149 (29.2%) were confirmed as BTT. Green and King index had the maximum sensitivity (67.1%) and Shine-Lal index had maximum specificity (91.1%) for diagnosing BTT. Green and King index also had the best Youden's index (43.9%).

Conclusion: Cell counter based formulas are cheaper, easy to calculate and reliable tools for screening BTT suspected cases which can be further confirmed by more specific tests like HPLC and electrophoresis. The authors found Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC) and Red Blood Cell (RBC) count as the most useful for selecting patients for more specific tests. The Green and King index was found to be most reliable for predicting BTT in the present study.

Keywords: Anaemia, Green and king index, High performance liquid chromatography, Shine-lal index

INTRODUCTION

The BTT originated from tropics and subtropics but is now found worldwide due to migration. It is associated with a defect in ß globin gene resulting in precipitation of excess alpha chains which cause mild haemolysis and ineffective erythropoesis. India lies in low prevalence region of BTT with an overall incidence of 2-3% except in isolated regions like Gujarat showing a high prevalence of 10-15% [1]. The disease is mostly asymptomatic in carrier state though anaemia can be aggravated during pregnancy or any other stressful condition. Also, iron overload, oligohydramnios and intrauterine growth retardation have been found to be associated with BTT [2].

Despite of mild nature of the disease, a need for screening arises as this disorder has a hereditary basis of inheritance and can lead to beta thalassemia major in the progeny if both parents carry the defective gene. BTT presents most commonly with a microcytic hypochromic blood picture along with target cells in the peripheral smear. Confirmation of the disease requires quantification of HbA2 by electrophoresis or high HPLC [2,3]. HPLC has become the most widely used technique for BTT diagnosis due to its specificity, rapidity and ability to detect as well as quantitate HbA2 in a single step. However, the technique is expensive. Therefore, most centres in developing and underdeveloped countries use different methods to triage patients for HPLC especially antenatal females. Electronic cell counter values of RBC indices (high RBC count and a low Mean Corpuscular Volume (MCV)) are helpful and are most commonly used in identifying suspected BTT cases for HPLC evaluation to reduce the unnecessary cost of investigations.

For the same purpose, various discriminating indices have been devised by different researchers to aid in identifying the cases which may be taken up for HPLC. All the currently available indices vary widely in their sensitivity and specificity and different authors have found different indices as superior to others for detection of BTT cases [3]. Moreover, many of these indices have been applied on Western population and data regarding their utility in Indian population is sparse where there is a need of eliminating other causes of microcytic hypochromic anaemia, especially IDA. Thus, the present study was undertaken primarily to compare the utility of 11 different indices available in literature in differentiating BTT cases from IDA in the study population which included - Mentzer, England Fraser, Srivastava, Shine-Lal, Ricerca, Red Cell Distribution Width Index (RDWI), Green and King, Mean Cell Haemoglobin Density (MCHD), Mean Density of Haemoglobin per Litre of blood (MDHL), Ehsani, Sirdah [4-13].

MATERIALS AND METHODS

This was an observational retrospective study. A total of 510 cases with MCHC anaemia presenting to Maluana Azad Medical College and Associated Hospitals over a period of one year (January 2017-January 2018) were analysed. Sampling was done to include all cases which presented in one year period that fulfilled the inclusion and exclusion criteria for the study. Patients with clinically suspected haemoglobinopathy or with microcytic hypochromic anaemia along with relative erythrocytosis were included in the study. After noting complete clinical details, data was collected regarding processing of the samples. All samples were processed in the following uniform manner: a 5 mL of venous blood was drawn under aseptic conditions out of which 2 mL was transferred in EDTA vial and 3 mL was transferred in plain vial. The sample of EDTA vial was used

for complete haemogram, peripheral blood smear examination, reticulocyte count and HPLC. The serum from plain vial was used for determining serum ferritin, serum B12 and serum folate by ELISA method. Cases with acute or chronic inflammation, transfusion in the past three months, macrocytic blood picture, serum B12 <200 pg/mL and serum folate <3 ng/mL were excluded from the study.

Haemogram was performed on a five part differential haematology analyser (sysmex XT 2000i) and RBC indices were recorded including MCV, MCH, MCHC, Red-cell Distribution Width (RDW) and RBC counts. Reticulocyte count was done by manual reticulocyte method using 1% new methylene blue and was counted in 1000 RBCs. HbA2 was quantified using Cation exchange-HPLC (BioRad laboratories, California, USA) using Variant II β-thal short program. An HbA2 value of 3.8-8% (according to manufacturer instructions) was used for confirming diagnosis of BTT. Cases with HbA2 more than 8% or any other variant Hb were also excluded from the study.

The cases confirmed as BTT on the basis of above parameters, were further analysed on basis of discriminator indices (Mentzer, England Fraser, Srivastava, Shine-Lal, Ricerca, RDWI, Green and King, MCHD, MDHL, Ehsani, Sirdah) which are used in literature for predicting BTT, were calculated and their differential utility were assessed by calculating the sensitivity, specificity, PPV, NPV [Table/Fig-1] [4-13]. Youden's index for each of the indices was also calculated using the formula = (sensitivity + specificity) - 100 [14].

S. no	Haematological index	Formula
1	Mentzer Index (MI) (1973) [4]	MCV/RBC
2	England JM and Fraser PM (E and F) (1973) [5]	MCV - (5 × Hb) - RBC - 3.4
3	Srivastava PC and Bevington JM (1973) [6]	MCH/RBC
4	Shine I and Lal S (S and L) (1977) [7]	MCV × MCV × MCH/100
5	Ricerca BM et al., (1987) [8]	RDW/RBC
6	Red Cell Distribution Width Index (RDWI) (1987) [9]	MCV × RDW/RBC
7	Green R and King R (G and K) (1989) [10]	MCV × MCV × RDW/Hb × 100
8	Mean Density of Haemoglobin per Litre (MDHL) (1999) [11]	(MCH/MCV) × RBC
9	Mean Cell Haemoglobin Density (MCHD) (1999) [11]	MCH/MCV
10	Ehsani MA et al., (2009) [12]	MCV - (10 × RBC)
11	Sirdah M et al., (2008) [13]	MCV - RBC - (3 × Hb)

[Table/Fig-1]: Different indices used for BTT.

STATISTICAL ANALYSIS

The statistical analysis was done using SPSS16 software. The mean values of RBC parameters (MCV, MCH, MCHC, RDW and RBC counts) were calculated and cut-offs were taken based on the ROC curves. The suspicious cases were put through HPLC analysis and diagnostic capabilities (sensitivity, specificity, NPV, PPV) of different indices were determined.

RESULTS

The study examined 510 cases of suspected haemoglobinopathy out of which 149 cases (29.2%) were confirmed as BTT. The rest of 361 cases were mostly IDA (333 cases; 65.29%) along with 28 cases (5.49%) of other haemoglobinopathies [Table/Fig-2].

The 149 cases of proven BTT were further analysed. The age group of BTT cases ranged from 1-67 years with a mean age of 15.32 years. There were slightly more males than females (M:F ratio-1.02:1). The mean haemoglobin value in beta thalassemia cases was 8.6 g/dL with 60.4% (90/149) of BTT cases having Hb less than 10 gm%. Relative erythrocytosis was seen in 65.1% cases as opposed to Non BTT group in which 53.5% showed a lower RBC count. The mean values of red cell indices are summarised in [Table/Fig-3].

Diagnosis	Cases Number (Percentage)
BTT	149 (29.2%)
IDA	333 (65.3%)
HbE+ß	9 (1.7%)
HbS+ß	1 (<0.1%)
HbD+ß	1 (<0.1%)
HbS	1 (<0.1%)
HbS trait	1 (<0.1%)
HbS+D	1 (<0.1%)
HbE homozygous	2 (<0.1%)
HbE heterozygous	5 (<0.1%)
HbD Punjab	1 (<0.1%)
HbJ Meerut	2 (<0.1%)
Hb Koln	2 (<0.1%)
HPFH	2 (<0.1%)

[Table/Fig-2]: Distribution of cases in the study.
BTT: Beta thalassemia trait; IDA: Iron deficiency anaemia; HPFH: Hereditary persistence of fetal haemoglobin

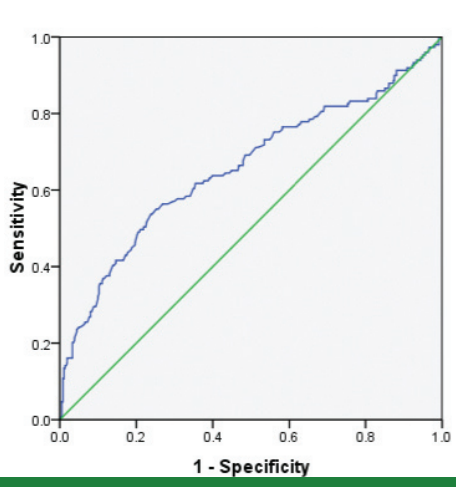
Index	MCV (fl)	MCH (pg/cell)	MCHC (g/dL)	RBC (cells/µL)	RDW (%)
Mean value	66.7	18.9	27.3	4.8	21.1

[Table/Fig-3]: Mean Red Cell Indices in BTT.

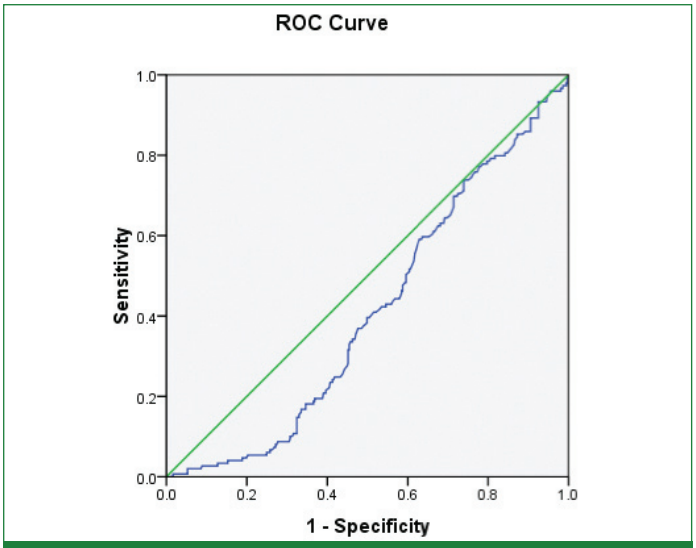
Based on Receiver Operating Characteristic (ROC) curve, MCV, MCH, MCHC and RBC counts were examined against the diagnosis of BTT. The Area Under Curve (AUC) was above 0.5 for MCH, MCHC and RBC counts. AUC of MCH, MCHC and RBC counts were 0.544, 0.650 and 0,653, respectively indicating that these values had some albeit low diagnostic ability for detection of BTT. However, AUC for MCV was 0.409, and therefore it was not found to have any diagnostic importance in this study. The cut-off values based on ROC curves were 16.05 pg for MCH, 27.35 g/dL, 19.5% for RDW and 5.20 million/mm^3 for RBC counts. For suspicion of BTT, the above values were regarded as cut-offs [Table/Fig-4-6].

S. no	Indices	AUC	Diagnostic value	Cut-off value
1	MCV	0.409	Negative	NA
2	MCH	0.544	Positive	16.05 pg
3	MCHC	0.650	Positive	27.35 g/dL
4	RBC counts	0.658	Positive	5.20 milion/µL
5	RDW	0.53	Positive	19.5%

[Table/Fig-4]: Cut-off Values of RBC indices based on ROC curve.



[Table/Fig-5]: RBC count ROC.



[Table/Fig-6]: MCV ROC.

The different indices for diagnosing BTT were examined. The sensitivity was found to be highest for Green and King (67.1%) followed by England Fraser (64%) index. The specificity was maximum in Shine-Lal (91.1%) and Sirdah (80.7%) indices. Green and King index had the best Youden's index (43.9%) revealing maximum predictability for BTT as shown in [Table/Fig-7]. The following rankings were found for the prediction of BTT based on Youden's index: Green and King > England Fraser > Sirdah > RDWI > Shine-Lal > Mentzer > Ehsani > Shrivastava > Ricerca > MCHCD > MDHL [4-13].

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Youden's index
Mentzer index (MI) (1973) [4]	36.7	80	69.1	50.9	16.7
England JM and Fraser PM (E and F) (1973) [5]	64	76.7	32.2	92.5	40.7
Srivastava PC and Bevington JM (1973) [6]	33.1	76.4	67.1	44.1	9.5
Shine I and Lal S (S and L) (1977) [7]	34.2	91.1	93.9	25.5	25.3
Ricerca BM et al., (1987) [8]	32.6	75.3	63.7	45.7	7.9
Red cell Distribution width (RDWI)(1987) [9]	51.8	78.7	46.3	88.3	30.5
Green R and King R (G and K) (1989) [10]	67.1	76.8	31.5	93.6	43.9
Mean Density of Haemoglobin per litre (MDHL) (1999) [11]	20.1	37.1	54.3	11.1	-42.8
Mean Cell Haemoglobin Density (MCHD) (1999) [11]	24.5	57.5	61.7	21.3	-18
Ehsani MA et al., (2005) [12]	33.6	78.7	67.7	49.3	12.3
Sirdah M et al., (2007) [13]	55.3	80.7	52.3	82.5	36

[Table/Fig-7]: Comparison of various indices [4-13].

DISCUSSION

India lies in the thalassemia belt region and it is one of the most common causes of microcytic hypochromic anaemia. In this study, 149 (29.2%) microcytic hypochromic cases were found to be BTT positive.

An RBC count of 5.20 million/µL (based on ROC curve) [Table/Fig-5] was observed in more than 65% of BTT cases. A 60.4% cases had a Hb value below 10 mg/dL. Hence, cases with low Hb and raised RBC counts were more indicative of BTT as compared to non BTT cases which had lower RBC counts in 53.5% of cases. These findings were concordant with observations of other authors [3,15,16].

The MCH values of 16.05 pg, RBC count of 5.20 milion/mm^3, RDW of 19.5% and MCHC value of 27.35 g/dL were most reliable according to ROC curves in predicting BTT. Several studies from Indian subcontinent have found almost similar results. Parthasarathy V, found MCH of 28 pg or less, RDW of 18% or less and RBC count of atleast 4.9 million/mm^3 to be most reliable [17]. Kotwal J et al., found RBC count of 4.9 million /mm^3 and RDW of 18% or less to be best indicative of BTT [18]. Rathod DA et al., also documented MCH of 18.8 pg, RBC count of 5.52 million/mm^3, haemoglobin below 10.3 g% and RDW of 15.58% to be the best predictor of BTT [3]. Hence, present study findings were in concordance with the above authors as regards to value of indices in predicting BTT.

In the present study, 11 different indices were compared to identify their utility in differentiating BTT from other causes of microcytic hypochromic anaemia. Various discriminator indices have been used from time to time to screen out BTT cases. All of them were based on combinations of RBC indices. The effectiveness of these indices has been tested by some authors in different populations. However, the results have been fluctuant and greatly depend on the sample size and population area selected,. For example, a study found Shine-Lal, Shrivastava and Mentzer indices were better than other indices to discriminate BTT in Indian subcontinent but other authors had conflicting results [19].

For all the 11 indices, sensitivity, specificity, NPV, PPV and Youden's index were calculated. Based on these parameters, the best formula found to predict BTT was Green and King index with a sensitivity of 67.1% and Youden's index of 43.9 followed by England Fraser index having sensitivity of 64% and Youden's index of 40.7. The MDHL and MCHD were of least significance in screening out BTT cases. In the present study the specificity was found to be best for Shine-Lal (91.1%) and Sirdah (80.7%) indices. So, based on above findings, the most specific index was Shine-Lal while Green and King index was the best for screening out cases of BTT considering its high sensitivity. Similar findings have been observed in other studies. Madan N et al., observed 97.9%, 88.7%, 89%, and 83.4% sensitivity in classic BTT cases in Shine-Lal, Mentzer, Shrivastava and England Fraser, respectively [19]. Rathod AK et al., also found Shine-Lal index with a sensitivity of 93.3% and Green and King with a maximum specificity of 92.8% [3]. However, these findings differed significantly from studies by some authors [20-23]. This could be because of differences in the prevalence of disease in different populations and regions which would affect the predictability of different indices.

Literature reports BTT cases to a variable extent. Tiwari AK et al., found 36% BTT cases to be present in 50 MCHC anaemia samples [23]. Rahim F and Keikhaei B, found this value to be 47.36% in 323 Iranian patients [21]. One of the Indian studies by Parthasarathy V et al., in 200 patients found the prevalence as 19.5% [17].

In the present study, Green and King was the best index for predicting BTT followed in decreasing order by England Fraser >Sirdah > RDWI > Shine-Lal > Mentzer > Ehsani > Shrivastava > Ricerca > MCHD > MDHL. Many authors have found similar results. According to a study by Ntaios G et al., the Green and King index was the most reliable index to predict BTT, as it had the highest sensitivity (75.06%), specificity (80.12%), and Youden's index (70.86%) for detecting BTT [24]. Urrechaga E et al., also found similar result for the Green and King index (Youden's index, 80.9%) [25]. A study found that Green and King index also differentiates Alpha thalassemia from IDA better than other indices [26].

However, none of the indices in the present study had 100% sensitivity and specificity or could predict BTT in all cases. Also, some cases of BTT may have normal or near normal Hb and normal cell counter values. These cases can still be missed by this approach. Nonetheless, cell counter based indices are an indispensable element for screening thalassemia cases especially in areas with economical constraints.

Limitation(s)

The main limitation of the study was low sample size and limited population area. Further studies with larger sample size are recommended to arrive at final conclusion.

CONCLUSION(S)

Cell counter based formulas are cheaper, easy to calculate and reliable tools for screening BTT suspected cases which can be further confirmed by more specific tests like HPLC and electrophoresis. The authors found MCH, MCHC and RBC count as the most useful for selecting patients for more specific tests. The Green and King index was found to be most reliable for predicting BTT in northern Indian population.

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