Original Research Article

Evaluation of Red Cell Indices and Discriminant Functions in the Detection of Beta Thalassemia Trait

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Abstract

Background: The heterozygous state of Beta-Thalassemia i.e. Beta-Thalassemia Trait (BTT) is usually not associated with any clinical symptoms and possesses abnormality in only a single Beta-Globin gene.

Materials and methods: The present study was undertaken from October 2015 to October 2017 in the Department of Pathology of Mahatma Gandhi Medical College and Hospital. In our study, a total of 100 subjects were included with mild or no anemia (Hemoglobin >8 gm/dl) who were referred to the central lab for hemoglobin screening. Hb A2 values were determined by Capillary Hemoglobin electrophoresis for all 48 (BTT) and 52 (Normal/non BTT) subjects. The complete hemogram was done by automated hematology analyzer. The values of red cell indices TRBC, MCV and MCH and six discriminative indices were evaluated for detection of β-thalassemia trait. The results were compiled in tabular form and bar diagram.

Results: In the present study, the patients in the BTT group had statistically significantly decreased mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) and increased TRBC count as compared to those in non BTT group. Among the six discrimination indices used highest sensitivity was found for Shine and Lal index (87.5%) followed by Mentzer index (79.16%). The highest specificity was found for E and F (88.46%) followed by RDWI (84.61%). Youden's index was highest for Mentzer index (61.85%) followed by RDWI (55.44%).

Conclusion: From this study, we thus conclude that although no screening test can diagnose β−Thalassemia Trait with 100% sensitivity or specificity, among the hematological parameters MCV, TRBC and MCH most efficiently discriminates β−Thalassemia Trait from other microcytic, hypochromic anemia. Mentzer index with CBC may be the simple, low cost, rapid and can be reliably

used as a screening test for thalassemia as a routine. However none of the formulas are 100% sensitive and specific.

Key words

Red Cell Indices, Beta Thalassemia Trait, Evaluation, Discriminant Functions.

Introduction

The heterozygous state of Beta-Thalassemia i.e. Beta-Thalassemia Trait (BTT) is usually not associated with any clinical symptoms and possesses abnormality in only a single Beta-Globin gene. The frequency of β−Thalassemia Trait in different regions of India is reported to be from <1% to 17% with an average of 3.3% [1].

Effective screening of population of β−Thalassemia Trait can drastically decrease the progression to homozygous Beta Thalassemia to enable the implication of efficient genetic counseling [2]. It is accounted that approximately 1.5% of the world's population are carriers of β-thalassemia gene, i.e. about 80-90 million people with a figure of 60,000 new carriers are born every year [3].

The region of Southeast Asia (which includes India, Thailand, and Indonesia) comprise of about 50% of the world's carriers i.e., around 40 million people and almost half of total number of homozygous births. On the other in the developed world which include Europe and United States of America jointly include around 10% to 13% of the world's carriers [4]. β-thalassemias are commonest monogenic diseases in which there is reduced rate of synthesis of β-globin chains leading to imbalanced globin chain synthesis [5].

Production of β-globins chains is impaired because of compound Heterozygous or Homozygous mutations in the β-globin gene. This leads to excess production of α-Globin chains which are incapable of forming a viable hemoglobin tetramer and hence precipitate in red cell precursors [6, 7]. It is vital to differentiate between β−thalassemia trait and iron deficiency anemia, so as to avoid unnecessary iron therapy which is contraindicated in β− Thalassemia Trait and further for the prevention of β−Thalassemia Major by genetic counseling. Through genetic counseling 90% reduction in birth rate of beta thalassemia major can be achieved [8].

Hemoglobin A2 estimation is necessary for definite diagnosis of β−Thalassemia Trait cases. Normally Hb A2 is less than 3.2% but in β−Thalassemia Trait cases it is more than 3.5% [9]. In remote areas where availability of modern, expensive methods for diagnosis is not there a simple morphological criterion has been advocated. Early detection of the trait in the antenatal patient and her spouse is important to prevent the birth of a homozygous thalassemia major child. However safe blood is available only for a small fraction and most transfusion dependent patients die from iron overload unless an oral, inexpensive iron chelation is made more widely available [10]. A red cell indices based analysis may complement areas with low health care resources where advanced investigations may not be generally available.

This study aimed at initial detection of β-thalassemia trait by red cell indices (TRBC, MCV, MCH) obtained on automated hematology analyzer requiring appropriate follow up to reduce unnecessary investigation costs and for genetic counseling. Thereafter mathematical calculation and evaluation of various discriminant functions obtained by RBC indices in detection of β-thalassemia trait.

Mentzer count - MCV/RBC count [11]
Srivastava index - MCH/RBC count [12]
Shine & Lal index - MCVxMCVxMCH/100 [13]
Green & King index - MCVxMCVxRDW/(Hbx100) [14]

Red Cell Distribution width index - MCVxRDW/RBC [15]
England & Fraser index - MCV-(5xHb)-RBC [16]

Sensitivity, Specificity, Positive Predictive Value, Negative Predictive Value was calculated.

Materials and methods

The present study was undertaken from October 2015 to October 2017 in the Department of Pathology of Mahatma Gandhi Medical College and Hospital. In our study, a total of 100 subjects were included with mild or no anemia (Hemoglobin >8 gm/dl) who were referred to the central lab for hemoglobin screening. Hb A2 values were determined by Capillary Hemoglobin electrophoresis for all 48 (BTT) and 52 (Normal/non BTT) subjects. The complete hemogram was done by automated hematology analyzer. The values of red cell indices TRBC, MCV and MCH and six discriminative indices were evaluated for detection of β-thalassemia trait. The results were compiled in tabular form and bar diagram.

Eligibility criteria were as follows

Inclusion criteria

- Hb >8 gm/dl
- HbA2 >3.5 (for β-thalassemia trait patients) HbA2 <3.5 (for non β-thalassemia trait patients).
- Patients with all age groups and both gender were included.
- Patients who had given consent.

Exclusion criteria

- History of recent blood transfusion or iron therapy.
- History of bleeding episodes previously.
- Patients who have other hemoglobinopathies.
- Patients with Thalassemia major.
- Patients who had not given consent.

Detailed medical history

a) Family history
b) Genetic history
c) Blood Transfusion history
d) Blood related infections (Viral and Bacterial)

Samples for analysis

Fresh EDTA blood samples were taken for analysis.

Investigations

a) Complete blood count (CBC)
b) Screening of hemoglobin by Capillary Hemoglobin Electrophoresis.

The blood counts including the Red blood cell (RBC) indices and Red cell distribution width (RDW) were done on Sysmex XS-800i-5 part analyzer working on impedance method. It detects Hb based on SLS hemoglobin detection method. BTT is diagnosed with Hb A2 > 3.5% while non BTT are those with Hb A2< 3.5% with normal hemoglobin electrophoretic pattern. All the subjects selected comprised of Hb>8% as there are high chances for these group of anemic patients to be missed for β-thalassemia trait in routine practice. In Mahatma Gandhi Hospital, hemoglobin A2 estimation is done by capillary hemoglobin electrophoresis.

Data analysis

Following parameters were taken from automatic hematological analyzer.
Hb, MCV, MCH, MCHC, TRBC, RDW.
HbA2 estimation was done by capillary electrophoresis in all patients.
The criteria to label the case as β-Thalassemia trait was Hb A2>3.5%.
Value of Hb A2 was noted and the case was classified as-
1) β-Thalassemia Trait-Present (BTT) [18]
2) β-Thalassemia Trait- not Present (non BTT)
Data was computed and the three RBC indices-Total RBC Count (TRBC), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) were evaluated for mean, standard deviation and unpaired t test. Red cell counter based formulae to differentiate BTT from Non BTT was as per **Table – 1**.

Table - 1: Red cell counter based formulae to differentiate BTT from Non BTT.

Index	Formula	BTT	Non BTT
Mentzer count [11]	MCV/RBC count	<13.0	>13.0
Srivastava [12]	MCH/RBC count	<3.8	>3.8
Shine & Lal [13]	MCVxMCVxMCH/100	<1530	>1530
Green & King [14]	MCVxMCVxRDW/(Hbx100)	<72	>72
RDWI [15]	MCVxRDW/RBC	<220	>220
England & Fraser [16]	MCV-(5xHb)-RBC	<0 (Negative)	>0 (Positive)

Table - 2: Sensitivity, Specificity, positive predictive value (PPV), negative predictive value (NPV), Youden's index of six discriminant indices.

Discriminant Indices	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	YI (%)
Mentzer Index	79.16	82.69	80.85	81.13	61.85
RDWI	70.83	84.61	80.95	75.86	55.44
Shine and Lal Index	87.5	51.92	62.68	81.81	39.42
Srivastava Index	70.83	80.76	77.27	75	51.60
Green and King Index	75	75	73.46	76.47	50
England and Fraser Index	64.58	88.46	83.78	73.01	53.04

All 100 patients were studied for six discrimination index which were calculated from RBC indices obtained from automated hematology analyzer. The Sensitivity, Specificity, Positive Predictive Value, Negative Predictive Value and Youden's Index of each discrimination index were calculated.

Sensitivity and specificity, positive and negative predictive values were calculated as follows.

Sensitivity: True positive / (true positive + false negative)

Specificity: True negative / (true negative + false positive)

Positive predictive value: True positive / (true positive + false positive)

Negative predictive value: True negative / (true negative + false negative)

Youden's index: (sensitivity + specificity) - 100

Results

Sensitivity, Specificity, positive predictive value (PPV), negative predictive value (NPV), Youden's index of six discriminant indices were as per **Table – 2**. Interpretation of Discriminant Indices according to Sensitivity was as per **Graph – 1**. Interpretation of Discriminant Indices according to Specificity was as per **Graph – 2**. Interpretation of Discriminant Indices according to Positive Predictive Value was as per **Graph – 3**. Interpretation of Discriminant Indices according to Negative Predictive Value was as per **Graph – 4**. Interpretation of Discriminant Indices according to Youden's Index was as per **Graph – 5**.

Discussion

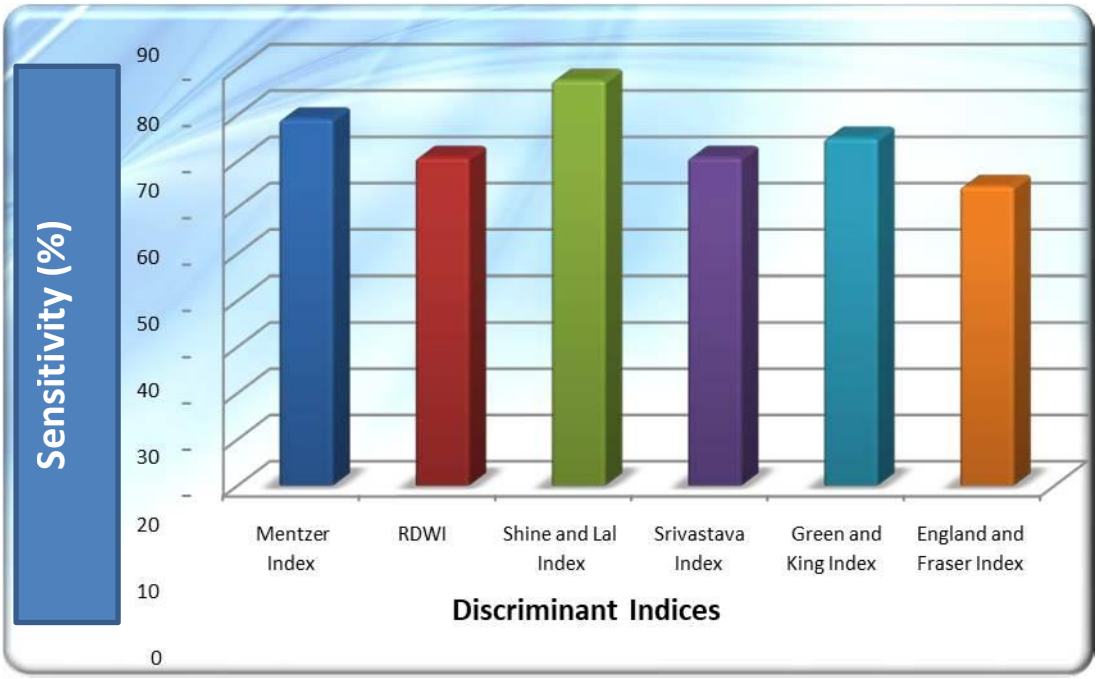
The various red cell indices offer a prompt and reliable method for effective screening of β−Thalassemia Trait. In addition to red cell indices - RBC count, MCV and MCH obtained from automated hematology analyzer and several formulae based on these Red cell indices has been used in an attempt for early identification of β− thalassemia trait. These formulae can be used as initial screening tools for early detection of β-Thalassemia Trait and to allow verification by standard tests like hemoglobin A2 estimation. This study showed a highly significant lower mean corpuscular volume (in fl) of 63.33 ± 9.70 in BTT group compared to 77.30±10.8 in the non BTT group.

Amna A, et al. [17] reported an average MCV of 65.2±5.75. Ehsani, et al. [18] found average MCV to be 62.02±4.57 in BTT group and

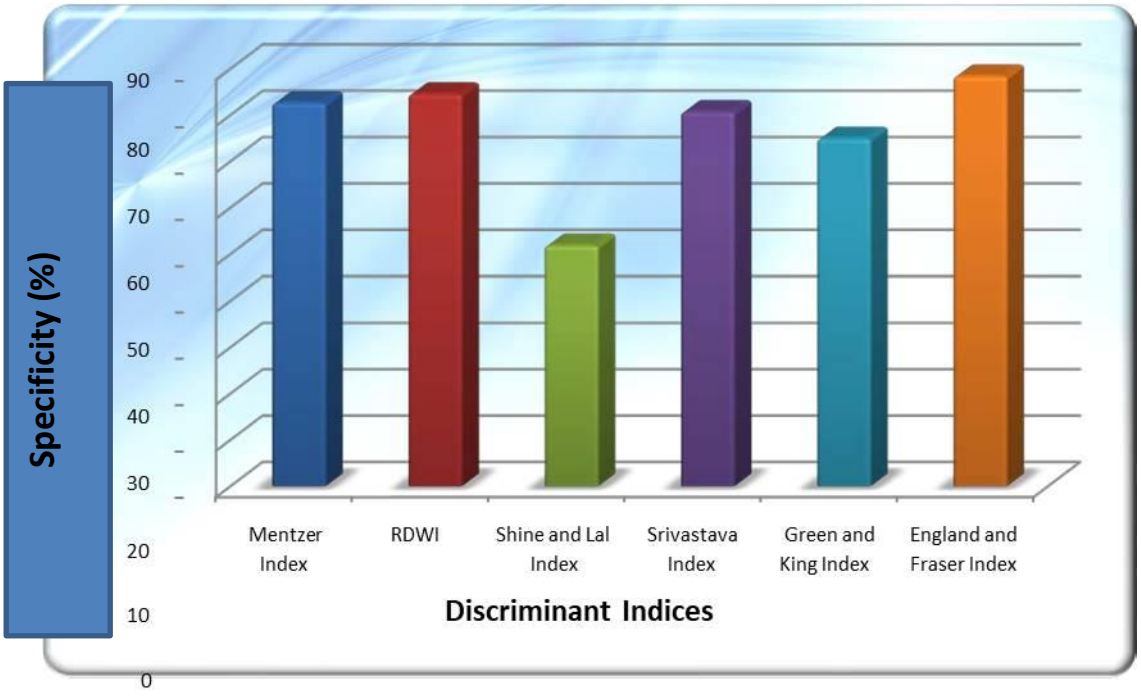
70.04±7.94 in the IDA group. Zahid, et al. [19] found MCV was 66.82±8.9 fl in BTT group. This study confirms that MCV values were low in BTT patients.

This study also revealed a highly significant lower MCH (pg) of 19.81± 2.59 in BTT group compared to 25.2±5.01 in the non BTT group. Ehsani, et al. [18] noted a MCH of 19.68±1.53 in the BTT group and 21.30±3.52 in the IDA group. Zahid, et al. [20] noted a MCH of 20.76±4.69 in BTT group. A lower MCH is seen in β-thalassemia trait. The results of this study matched most other studies.

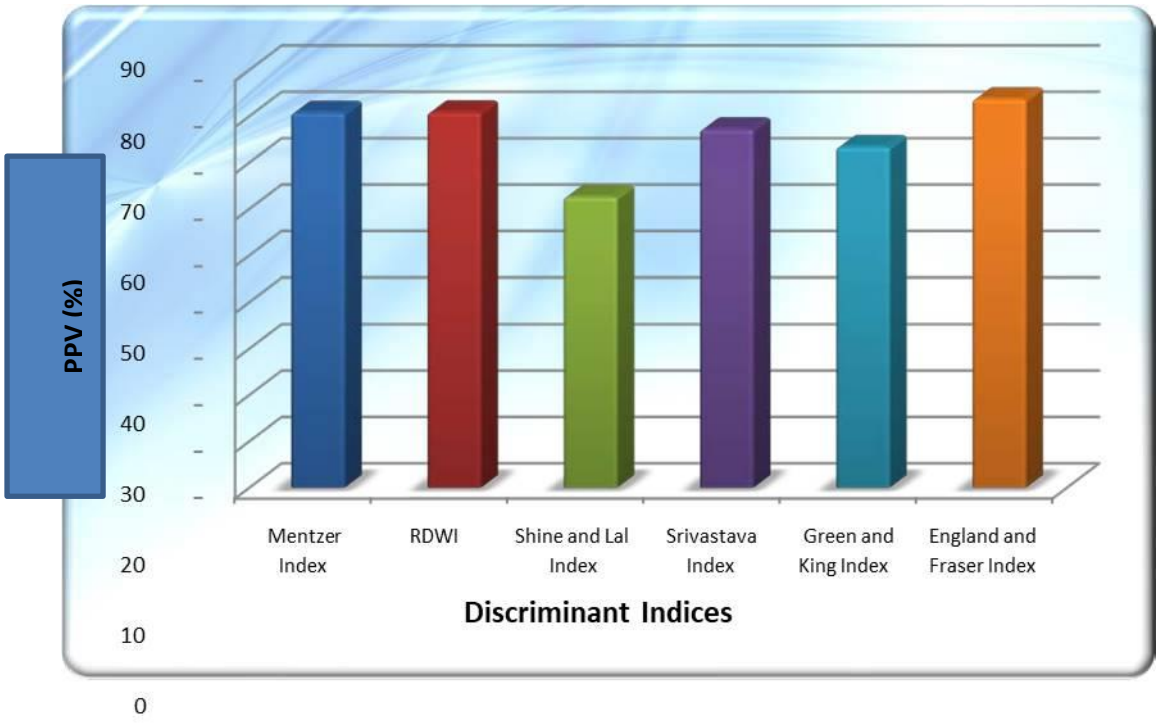
Graph - 1: Interpretation of Discriminant Indices according to Sensitivity.



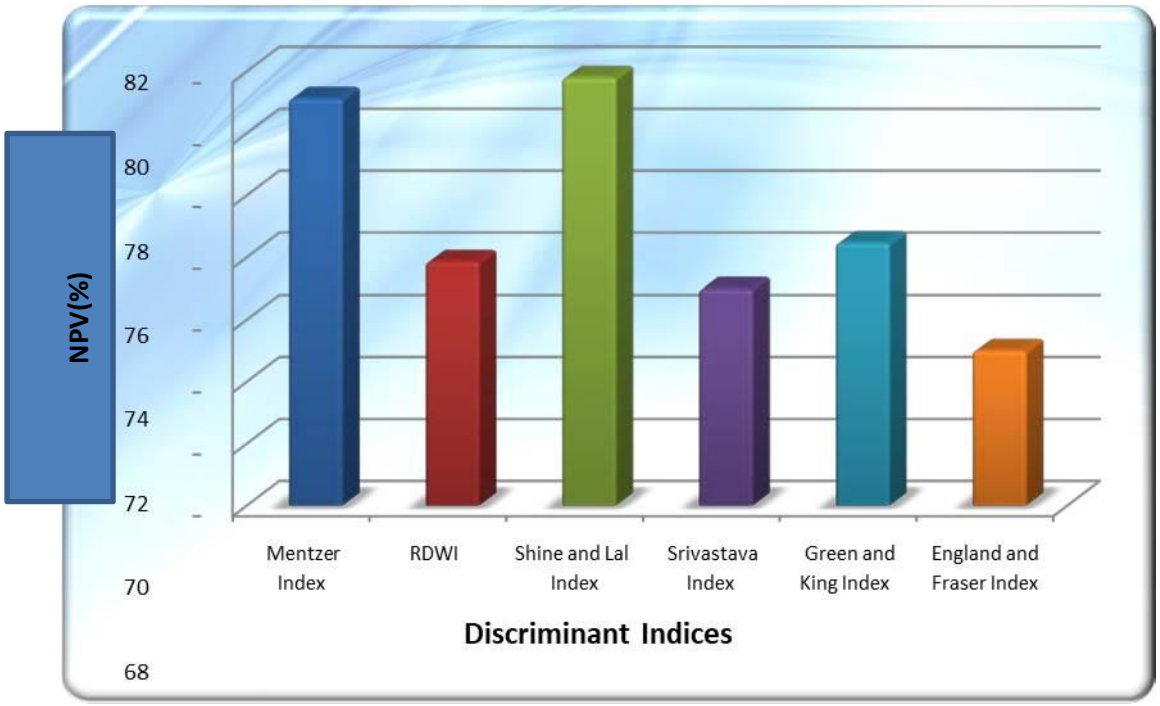
Graph - 2: Interpretation of Discriminant Indices according to Specificity.



Graph - 3: Interpretation of Discriminant Indices according to Positive Predictive Value.



Graph - 4: Interpretation of Discriminant Indices according to Negative Predictive Value.

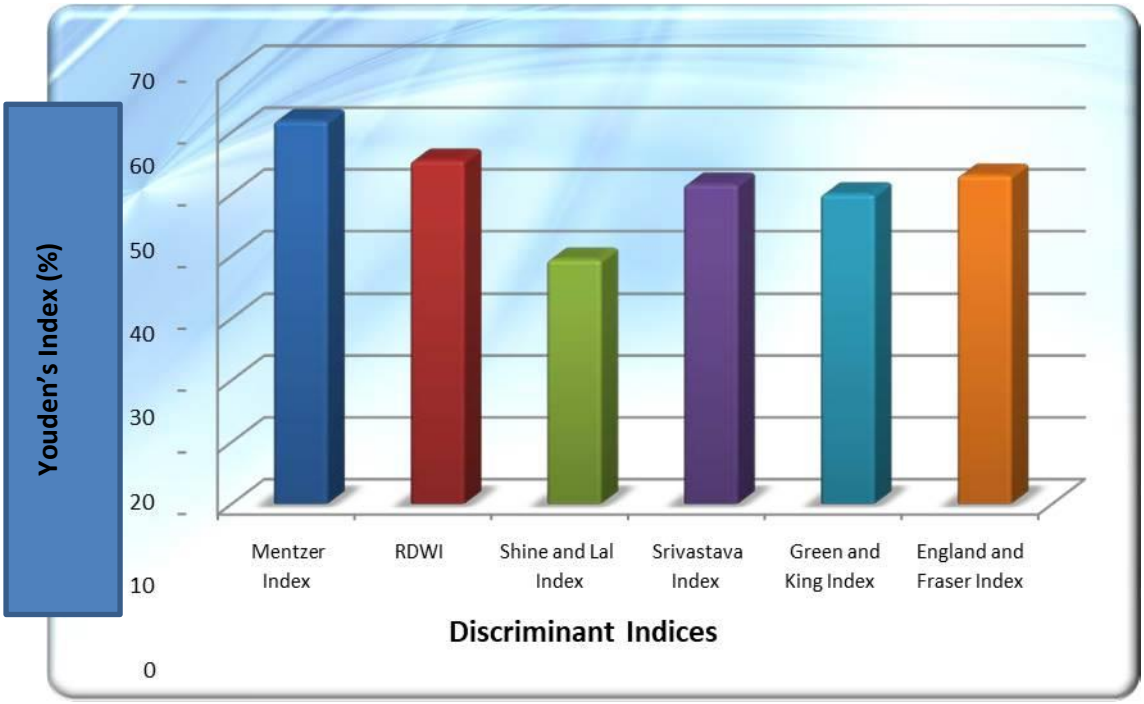


This study showed a highly significant higher RBC count (above 5.0x106/µl) with a mean of 5.56±1.016 in BTT group as compared to 4.73±0.676 in the non BTT group. Ehsani, et al. [18] reported a higher Sensitivity and Specificity of 98.1% and 86.2% respectively. Ntaios, et al. [20] reported a Sensitivity and Specificity of 64.34% and 99% respectively.

The values of MCV less than 76 fl [21] and MCH less than 26 pg [21] and Total Red Blood Cell count(TRBC) > 5million/cu.mm [22] are

suggested to be associated with a high probability of BTT. In 2013 Bhukhanvala D, et al. found values of MCV 78.0 fl or less, MCH 28 pg or less, for BTT diagnosis [23]. However, in 2012 Parthasarathy in India concluded that values of MCV below 76 fl, RBC count at least $4.9\times10^6/mm^3$, and to be associated with a high probability of BTT [24]. In the present study, six discriminant functions were used and Sensitivity and Specificity, Positive predictive value and Negative predictive value for each discrimination index is calculated. In addition Youden's index is also calculated for each discriminant function.

Graph - 5: Interpretation of Discriminant Indices according to Youden's Index.



Erythrocyte Indices

1) Mentzer Index

Sensitivity (Sn) and specificity (Sp) of the index were 79.16% and 82.69% respectively, while the positive predictive value (PPV) and negative predictive value (NPV) were 80.85 and 81.13 respectively. Overall the Youden's index was 61.85. Similarly other studies done by Sehgal, et al. [25] showed Youden's index of 64% for Mentzer index, 64.5% by Matos JF, et al. [26], 63.2% by TP, SA, et al.[27] and 60% by Okan V, et al. [28].

2) RDWI

Sensitivity (Sn) and specificity (Sp) of the index were 70.83% and 84.61% respectively, while the positive predictive value (PPV) and negative predictive value (NPV) were 80.95 and 75.86 respectively. Overall the Youden's index was 55.44. In 2009, Nesa, et al. [29] reported a sensitivity of 80.7% and specificity of 84.7% in detection of β− Thalassemia Trait. These results are consistent with the findings of Demir, et al. [30] and Sirdah, et al. [31].

3) England Fraser Index

Sensitivity (Sn) and specificity (Sp) of the index were 64.58% and 88.46% respectively, while the positive predictive value (PPV) and negative predictive value (NPV) were 83.78 and 73.01 respectively. Overall the Youden's index was 53.04. Ehsani, et al. [18] reported a higher Sensitivity and Specificity of 69.5% and 99.2% respectively. Ntaios, et al. [20] reported a Sensitivity and Specificity of 64.07% and 99% respectively. TP, SA, et al. [27] calculated positive predictive value of 91.3% for England

and Frazer index which was slightly higher than the present study.

4) Srivastav Index

Sensitivity (Sn) and specificity (Sp) of the index were 70.83% and 80.76% respectively, while the positive predictive value (PPV) and negative predictive value (NPV) were 77.27 and 75 respectively. Overall the Youden's index was 51.60. In 2010, Niazi M, et al. [32] showed that percentage of correctly diagnosed cases for Srivastava index was 82.37%. Demir A, et al. [30] found 67% of the cases to be correctly diagnosed by Srivastava index.

5) Green King Index

Sensitivity (Sn) and specificity (Sp) of the index were 75% and 75% respectively, while the positive predictive value (PPV) and negative predictive value (NPV) were 73.46 and 76.47 respectively. Overall the Youden's index was 50. Okan V, et al. [28] reported a sensitivity of 83% and sensitivity of 79.5% by Sehgal, et al. [25] which was slightly higher than the present study.

6) Shine and Lal Index

Sensitivity (Sn) and specificity (Sp) of the index were 87.5% and 51.92% respectively, while the positive predictive value (PPV) and negative predictive value (NPV) were 62.68 and 81.81 respectively. Overall the Youden's index was 39.42. In 2015 Kunal Sehgal, et al. [25] gave sensitivity of 94.9% by Shine and Lal index which is slightly more than the present study, 91% by Okan V, et al. [28], followed by 72% by Niazi M, et al. [32]. In 2009, Ehsani, et al. [18] also reported Mentzer index as the best discrimination index followed by RBC count and England Fraser. In 2007, Ntaios, et al. [20] found Green King index to be the best index in their study followed closely by England Fraser, RBC count, and Mentzer index.

In the present study the Mentzer index had the best overall accuracy (Youden's index 61.85) followed by RDWI (YI of 55.44), England and Fraser (YI of 53.04), Shrivastav index (YI 51.60), G and K index (YI 50) Shine and Lal index (Y.I 39.42) in decreasing order. Highest sensitivity was of Shine and Lal index (87.5%) followed by Mentzer index (79.16%), G and K index (75%), RDWI (70.83%), Srivastav index (70.83%), and E and F (64.58%) in decreasing order. The specificity was highest of E and F (88.46%) followed by RDWI (84.61%), Mentzer index (82.69%), Srivastav index (80.76%), G and K (75%), Shine and Lal (51.92%) in decreasing order. Positive predictive value was found highest for E and F (83.78%) followed by RDWI (80.95%), Mentzer index (80.85%), Srivastav index (77.27%), G and K (73.46%) and Shine and Lal (62.68%) in decreasing order. Negative predictive value was found highest for Shine and Lal (81.81%) followed by Mentzer index (81.13%), G and K (76.47%), RDWI (75.86%), Srivastav index (75%) and E and F (73.01%) in decreasing order.

Conclusion

Automated cell counters provide a rapid, technically reliable method for screening of BTT cases. Red blood cell indices derived from them are major contributors for extensive screening and appropriate detection of beta thalassemia trait.

In the present study, the patients in the BTT group had statistically significantly decreased mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) and increased TRBC count as compared to those in non BTT group. From this study, we thus conclude that although no screening test can diagnose β− Thalassemia Trait with 100% sensitivity or specificity, among the hematological parameters MCV, TRBC and MCH most efficiently discriminates β−Thalassemia Trait from other microcytic, hypochromic anemia. Among the six discrimination indices used highest sensitivity was found for Shine and Lal index (87.5%) followed by Mentzer index (79.16%). The highest specificity was found for E and F (88.46%) followed by RDWI (84.61%). Youden’s index was highest for Mentzer index (61.85%) followed by RDWI (55.44%). The

present study gives us an idea that the Mentzer index with CBC may be the simple, low cost, rapid and can be reliably used as a screening test for thalassemia as a routine. However none of the formulas are 100% sensitive and specific.

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