Study of discriminant factor M/H ratio in screening for β thalassemia trait

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Abstract
Introduction: Beta Thalassemia syndrome is a group of hereditary disorders characterized by genetic deficiency in the synthesis of beta globin chains located on chromosome 11. A major diagnostic challenge is to differentiate between mild microcytic hypochromic anaemia due to BTT from other causes such as iron deficiency (IDA), sideroblastic anaemia etc. The microcytic to hypochromic ratio (M/H ratio) is one of the simplest discriminant functions which is based on the fact that in Iron deficiency anaemia the RBCs are more hypochromic as compared to thalassemia trait. The present study was carried out in our diagnostic centre with the aim of evaluating the M/H ratio for screening patients for BTT.

Materials and Methods: This was a retrospective study carried out on a total of 200 patients between January to April 2018 coming to our diagnostic centre for complete blood count and Hb electrophoresis for evaluating the M/H ratio to screen for BTT. HbA2 > 3.5% was considered to be diagnostic of BTT. M/H ratio as an indicator of differentiating between BTT and IDA was compared with other discriminant factors like England & Fraser, Mentzer Index, Shine & Lal Index and Shrivastava Index.

Results: There were 73 (36.5%) male and 127 (63.5%) females with a M:F ratio of 0.57:1. Out of 200 patients, 22 (11%) patients were diagnosed as BTT. Of these M/H ratio was <0.9 in 5 (22%) patients while >0.9 in 11 (77.27%) patients. England and Fraser index had highest specificity (94%) with a sensitivity of 23%. Shine and Lal had a specificity of 39% and sensitivity of 95%, Mentzer had a specificity of 83% and sensitivity of 45%, Shrivastava had sensitivity and specificity of 36% and 78% respectively while M/H ratio had specificity (70%) and sensitivity (77%).

Conclusion: M/H ratio provided by Advia 2120 hematology analyzer is as effective as preliminary screening tool for selection of samples for HbA2 estimation. It is a rapid, automated formula provided without any additional cost to the patient.

Keywords: M/H ratio, BTT, IDA, Discriminant factor.

Introduction
Beta thalassemia syndrome is a group of hereditary disorders characterized by genetic deficiency in the synthesis of beta globin chains located on chromosome 11. The homozygous condition is called thalassemia major and is a severe transfusion dependent condition while heterozygotes have minor symptoms related to anaemia and are called Beta thalassemia trait (BTT) or thalassemia minor.1,2 No specific treatment is needed for BTT patients. Thalassemias are more common in Mediterranean region, Africa, South East Asia with a prevalence rate of as high as 10% in these areas.

Thalassemia minor patients have a mild microcytic, hypochromic anaemia and are usually asymptomatic and do not have major morbid symptoms. However, a major diagnostic challenge is to differentiate between mild microcytic hypochromic anaemia due to BTT from other causes such as iron deficiency (IDA), sideroblastic anaemia etc.

A complete blood picture often gives an indication suggesting BTT due to presence of target cells and microcytic hypochromic RBCs on peripheral blood smear with an increased RBC count. An elevated HbA2 level on Hb electrophoresis is confirmatory.

There are many discriminant formulas which have been published for distinguishing the cause of microcytosis as thalassemia based on RBC parameters.3-7 Some of these Discriminant functions are simple to use while some require mathematical calculations and are complicated.8 The microcytic to hypochromic ratio (M/H ratio) is one of the simplest discriminant functions which is based on the fact that in Iron deficiency anaemia the RBCs are more hypochromic as compared to thalassemia trait.8-10

The Advia 2120 5-part hematology analyzer (Siemens) provides the percentage of microcytic to hypochromic cells. The M/H ratio is calculated by dividing the percentage of microcytic RBCs to the percentage of hypochromic RBCs and is automatically provided along with other CBC parameters by Advia 2120 hematology analyzer. A ratio of >0.9 is highly suggestive of BTT while ratio of <0.9 is suggestive of IDA.

The present study was carried out in our diagnostic centre with the aim of evaluating the M/H ratio for screening patients for BTT.

Materials and Methods
This was a retrospective study carried out on a total of 200 patients between January to April 2018 coming to our diagnostic centre for complete blood count and Hb electrophoresis for evaluating the M/H ratio to screen for BTT.

The patients of both sexes and all age groups were included in the study. The patients were divided into 0-10, 11-20, 21-30, 31-40, 41-50, 51-60 and more than 60 years of age in both the sexes. Venous blood samples
were collected into K2EDTA tubes and were subjected to complete blood count on Advia 2120 within one hour of sample collection.

The Advia analyzer was calibrated and 3 levels of controls were run before analyzing the specimens. HbA2 was assayed on BIO-RAD D10 HPLC electrophoresis analyzer (Bio-Rad Laboratories Hercules, CA, USA). All patients with Hb <12 gm% (WHO criteria for anaemia) were included in the study. HbA2 > 3.5% was considered to be diagnostic of BTT. Differential values for different discriminant indices were applied as defined in their original reports like Mentzer Index, Shine & Lal Index, England & Fraser Index and Shrivastava index.11-14

Results

A total of 200 patients of both sexes and all ages were included in the study. There were 73 (36.5%) male and 127 (63.5%) females with a M:F ratio of 0.57:1.

Maximum patients 76 (38%) were in the 21 – 30 years of age group followed by 42 (21%) in 0-10 and 35 (17.5%) in 31 – 40 years of age and 35 (17.5%) in 11-20 years of age group. (Table 1)

The Hb% concentration between 9-11 gm% was found in 44 (22%) patients, followed by 43 (21.5%) between 7.1 – 9.0 gm%. Maximum patients had Hb >11 gm% i.e. 66(30.5%). Only 20 patients (10%) had Hb below 5 gm% (Table 2) RBCs count <5.0 million/cumm was found in 157 patients (78.5%) while RBC count >5.0 million/cumm was found in 43 (21.5%) patients. An interesting observation was that 13 BTT cases had RBC count below 5 million/ cumm as compared to 9 who had RBC count greater than 5.0 million/cumm (Table 3). Out of 200 patients, 22 (11%) patients were diagnosed as BTT. Of these M/H ratio was <0.9 in 5 (22%) patients while >0.9 in 11 (77.27%) patients (Table 4).

When M/H ratio was divided into different categories it was observed that 50% patients of BTT had M/H ratio above 1.80. 13.6% had M/H ratio between 1.21 – 1.50 and 4.54% had M/H ratio between 1.51 – 1.80.

The mean age was 22.9 ± 12.9 years. The mean Hb was 9.3 ± 3.2 gm%. Mean RBC count was 4.2 ± 1.0 x 10^12/ul, mean PCV was 30.4 ± 9.5%, Mean MCV was 73.6 ± 20.0 fl, Mean M/H ratio was 0.89 ± 0.9 and Mean HbA2 was 2.8 ± 1.0%.

M/H ratio as an indicator of differentiating between BTT and IDA was compared with other discriminant factors like England & Fraser, Mentzer Index, Shine & Lal Index and Shrivastava Index (Table 5).

Table 1: Showing demographic data of patients

| Age in years | Male | Female | Total | %    |
|--------------|------|--------|-------|------|
| 0 - 10       | 29   | 13     | 42    | 21.0 |
| 11.0 - 20    | 12   | 23     | 35    | 17.5 |
| 21 - 30      | 21   | 55     | 76    | 38.0 |
| 31 - 40      | 6    | 29     | 35    | 17.5 |
| 41 - 50      | 2    | 4      | 6     | 3.0  |
| 51 - 60      | 2    | 3      | 5     | 2.5  |
| > 60         | 1    | 0      | 1     | 0.5  |
| **Total**    | 73 (36.5%) | 127 (63.5%) | 200 &nbsp; |

Table 2: Showing distribution of patients according to hematological parameters

| Hb (gm%)   | Male | Female | Total | %    |
|------------|------|--------|-------|------|
| <5.0       | 8    | 12     | 20    | 10.0 |
| 5.1 - 7.0  | 16   | 16     | 32    | 16.0 |
| 7.1 - 9.0  | 10   | 33     | 43    | 21.5 |
| 9.1 - 11.0 | 11   | 33     | 44    | 22.0 |
| >11.0      | 28   | 33     | 61    | 30.5 |
| **Total**  | 73   | 127    | 200   |      |

| RBC Count | Male | Female | Total | %    |
|-----------|------|--------|-------|------|
| <5.0      | 44   | 113    | 157   | 78.5 |
| >5.0      | 29   | 14     | 43    | 21.5 |
| **Total** | 73   | 127    | 200   |      |

Table 3: Showing distribution of patients according to RBC count

| RBC Count | Normal | Thalassemia Minor |
|-----------|--------|-------------------|
| <5.0      | 144    | 13                |
| >5.0      | 34     | 9                 |
| **Total** | 178 (89%) | 22 (11%) |
Table 4: Showing distribution of patients according to micro/hypo ratio

| Micro/Hypo Ratio | Normal | Thalassemia Minor |
|------------------|--------|-------------------|
|                  | Total Number | % | Total Number | % |
| <0.9             | 124 | 69.6 | 5 | 22.7 |
| >0.9             | 54 | 30.3 | 17 | 77.27 |
| Total            | 178 | 22 |

| Micro/Hypo Ratio | Thalassemia Minor | Normal |
|------------------|-------------------|--------|
|                  | Total Patients | Percent | Total Patients | Percent |
| <=0.2            | 2 | 9.09 | 43 | 24.1 |
| 0.3 - 0.60       | 0 | 0 | 45 | 25.2 |
| 0.61 - 0.90      | 3 | 13.6 | 36 | 20.4 |
| 0.91 - 1.20      | 2 | 9.09 | 27 | 15.1 |
| 1.21 - 1.50      | 3 | 13.6 | 8 | 4.49 |
| 1.51 - 1.80      | 1 | 4.54 | 5 | 2.8 |
| > 1.80           | 11 | 50 | 14 | 7.86 |
| Total            | 22 | 178 |

Table 5: Comparison of discriminant functions for identifying thalassemia in patients

| Discriminant function | Formula | Optimal Cut off | T.Minor | Normal HbA2 | Sensitivity | Specificity |
|-----------------------|---------|-----------------|---------|-------------|-------------|-------------|
| England & Fraser      | MCV-RBC- (5xHb)-3.4 | 0 | 5 | 17 | 11 | 167 | 23% | 94% |
|                       |         | <13 | >13 | <13 | >13 |         |         |         |         |
| Mentzer               | MCV/RBC | 13 | 10 | 12 | 31 | 147 | 45% | 83% |
|                       |         | <1530 | >1530 | <1530 | >1530 |         |         |         |         |
| Shine & Lal           | MCV²xMCH/100 | 1530 | 21 | 1 | 108 | 70 | 95% | 39% |
|                       |         | <3.8 | >3.8 | <3.8 | >3.8 |         |         |         |         |
| Srivastava            | MCH/RBC | 3.8 | 9 | 13 | 40 | 138 | 36% | 78% |
|                       |         | >0.9 | <0.9 | >0.9 | <0.9 |         |         |         |         |
| M/H Ratio             | MIC/HPO | 0.9 | 17 | 5 | 54 | 124 | 77% | 70% |

Table 6: Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and Youden's index to discriminate between BTT and normal HbA2

| Indices | Sensitivity | Specificity | PPV | NPV | Youden Index (sensitivity+specificity)-100 |
|---------|-------------|-------------|-----|-----|------------------------------------------|
| Mentzer |             |             |     |     |                                          |
| BTT     | 45%         | 83%         | 24.4% | 92.5% | 28%                                      |
| Normal HbA2 | 83%     | 45%         | 92.5% | 24.4% |                                          |
| Shine & Lal |         |             |     |     |                                          |
| BTT     | 95%         | 39%         | 16.3% | 98.6% | 34%                                      |
| Normal HbA2 | 39%     | 95%         | 98.6% | 16.3% |                                          |
| Srivastava |         |             |     |     |                                          |
| BTT     | 36%         | 78%         | 18.4% | 91.4% | 14%                                      |
| Normal HbA2 | 78%     | 36%         | 91.4% | 18.4% |                                          |
| England & Fraser |         |             |     |     |                                          |
| BTT     | 23%         | 94%         | 31.3% | 90.8% | 17%                                      |
| Normal HbA2 | 94%     | 23%         | 90.8% | 31.3% |                                          |
| M/H Ratio |         |             |     |     |                                          |
| BTT     | 77%         | 70%         | 23.9% | 3.9% | 7%                                       |
| Normal HbA2 | 70%     | 77%         | 3.9% | 23.9% |                                          |

Table 6: Shows the sensitivity, specificity, PPV, NPV and Youden Index of all discriminant factors.

**Discussion**

Anaemia is the most common presenting symptom of a variety of diseases. Iron deficiency anaemia and beta thalassemia trait being common causes of anaemia. However, differentiating between IDA and BTT is a challenging problem and has important
clinical implications because both of these have different causes, prognosis and treatment. A missed case of BTT may have potential homozygous offspring. Hence, it is very crucial to have a high index of suspicion for BTT based on CBC parameters so that it can be diagnosed and proper counseling of family members can be done. Many discriminant indices have been proposed to differentiate and screen for IDA and BTT but none has 100% specificity or sensitivity.

An ideal discriminant index should have high sensitivity and specificity. A combination of two or more CBC parameters in discriminant indices can lead to a great improvement in screening of anaemic patients for BTT.

In recent years several studies have been carried out to assess the usefulness in the differential diagnosis of BTT by using M/H ratio.

In IDA, RBCs are produced which have decreased Hb concentration and high hypochromic cell population while in BTT, microcytes are generally smaller with more preserved Hb concentration and is characterized by increase in RBC% due to chronic increase in erythropoiesis.

In our study, we found that England and Fraser index had highest specificity (94%) with a sensitivity of 23%. Shine and Lal had a specificity of 39% and sensitivity of 95%, Mentzer had a specificity of 83% and sensitivity of 45%, Srivastava had sensitivity and specificity of 36% and 78% respectively while M/H ratio had specificity (70%) and sensitivity (77%).

In the study conducted by Eloisa et al, Shine and Lal provided the best sensitivity (100%) but low specificity (22.8%). Srivastava index had a good specificity (91.4%) but a sensitivity of 86.8%. In their study the M/H ratio shared a sensitivity of 99.2% and specificity of 77.1%.

In the study by A. Vehapoglu et al, they found Mentzer index to be most reliable index with the highest sensitivity of 98.7% and specificity of 82.3%.

Al Fadhli et al in their study concluded that England and Fraser index had the highest Youden index value (98.2%) for differentiating between IDA and BTT.

In a study conducted by Ehsani et al in 2009, Mentzer Index (90%) was found to be the best index for differentiating IDA and BTT. Similar results indicating Mentzer Index to be highly sensitive (90.9%) was observed by Ghafouri et al.

A study by Kabir AL et al, however showed 96% sensitivity for M/H ratio with a predictive value of 90.4% in screening for BTT. We observed that with a cut off value of 1.8 for M/H ratio, we could diagnose BTT with maximal diagnostic sensitivity but at expense of specificity.

Our study. However has some limitations as the inclusion criteria may be different from other studies as our aim was to investigate patients with microcytosis for BTT rather than for IDA. Moreover, we included subjects with normal Hb also who were registered for Hb electrophoresis as a part of family studies. Moreover, our sample size is also small which may have lead to difference in sensitivity and specificity of M/H ratio as well as Youden index in our study as compared to other studies.

Conclusion
Several discriminant indices have been in use to differentiate between IDA & BTT and M/H ratio provided by Advia 2120 hematology analyzer is as effective as preliminary screening tool for selection of samples for HbA2 estimation. It is a rapid, automated formula provided without any additional cost to the patients. Large prospective population studies are needed to assess the efficiency of M/H ratio for screening patients with BTT.

Conflict of Interest: None

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