

UTILITY OF ERYTHROCYTE INDICES FOR SCREENING OF β – THALASSEMIA TRAIT IN PREGNANT WOMEN ATTENDING ANTENATAL CLINIC

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ABSTRACT

Background: This study was undertaken to see the utility of erythrocyte indices for screening of beta thalassemia trait in pregnant women, as these indices are based on complete blood count reports which are routinely available and nowadays generated by automated hematology analyzer. **Material and methods:** The study was a cross sectional in which the complete blood count report of 300 pregnant women attending antenatal clinic mahila chikitsalaya, sms medical college, Jaipur with microcytosis (MCV <80fl) was analyzed. Four red blood cell indices namely – Mentzer index, England Fraser index, RBC count & Green king index were calculated for all patients from the complete blood count (CBC) reports. Among these, for those patients who were suspected to have beta thalassemia trait on the basis of the red blood cell indices, Hb A2 estimation by high performance liquid chromatography (HPLC), serum iron, total iron binding capacity (TIBC) & serum ferritin was done. The collected data was analyzed statistically and the sensitivity & specificity of the four red blood cell indices was calculated & compared according to Youdens test. **Results:** In our study the patients in the beta thalassemia trait group had significantly decreased mean corpuscular volume(MCV), mean corpuscular hemoglobin(MCH)and packed cell volume(PCV) compared to those in non beta thalassemia group. Mentzer index was the best among the four discrimination indices used. Green king index and Mentzer index, when both indices are combined this yield is more informative. **Conclusion:** Mentzer index is a useful screening test for detection of beta thalassemia trait.

Key words: Beta thalassemia trait, Red blood cell indices, Screening.

INTRODUCTION:

Thalassemia is one of the commonest single gene disorder representing a major health burden in India and in the world. They are a group of autosomal recessive disorders where there is an inhibition in the production of α or β globin chains of hemoglobin resulting in varying levels of anemia. (1)

It is estimated that more than 200 million people are carriers of the beta thalassemia gene in the

world and about 30 million of them are in India. Every year about 10,000 children are born with thalassemia major in India accounting for 10% of thalassemia major births world wide. (2, 3)

The frequency of beta thalassemia trait has variously been reported as <1% to 17% and with an average of 3.3%. Based on this figure an

estimate of 6000-7500 homozygous births of beta thalassemia has been made.(4)

Conventional therapy of beta thalassemia major (BTM) is life-long and places a significant load on blood transfusion services and finances. Prevention of birth of children with BTM would thus spare a lot of distress, effort and expenses for the families involved and for society. The key requirement for this is identification of couples at risk of giving birth to children with BTM.

The need, therefore, is for a simple, low cost, rapid and reliable screening test. Various screening parameters that are available include peripheral blood smear examination, red cell osmotic fragility test (NESTROFT), free red cell porphyrins & red cell indices.(5)

Complete blood counts provided by a routine automated blood counter and the red blood cell indices derived from them are major contributors for extensive screening & appropriate detection of beta thalassemia trait. They are routinely done now a day in all patients even at the periphery, and they need no extra cost or resource.

Health care providers usually do not give much importance to the red cell indices in complete blood count. Hence beta thalassemia is usually not suspected. These indices utilize mainly four different parameters from the complete blood count namely – hemoglobin, total red blood cell, mean corpuscular volume and red cell distribution width. Different studies have shown different indices to be superior to the rest in predicting beta thalassemia trait.(6)

So this study aims to detect beta thalassemia trait in pregnant women attending antenatal clinic on the basis of red blood cell indices and its confirmation by high performance liquid chromatography thereby finding the sensitivity, specificity & validity of the various red blood cell indices.

Those pregnant women found beta thalassemia trait positive by HPLC, their husbands were also investigated for beta thalassemia trait by HPLC.

MATERIAL & METHODS

The study was a cross sectional in which the complete blood count report of 300 pregnant women attending antenatal clinic mahila chikitsalaya, sms medical college, Jaipur with microcytosis (MCV <80fl) was analyzed after approval from institutional ethics committee .

Pregnant willing women age ≥ 18 years to ≤ 40 years attending antenatal clinic with microcytosis (MCV<80) were included in this study, while female having either anemia of chronic disease, other hemoglobinopathies, dimorphic anemia, well defined causes of anemia excluded from this study.

Written informed consent was taken from all subjects enrolled in the study. General physical examination (nutritional state and per abdominal examination) was done and family history, history of blood transfusion was taken and all patient enrolled in this study were investigated according to proforma chart.

Four red blood cell indices were calculated which were based on the hematological parameters derived from complete blood count reports.

The indices used were Mentzer index (7), England-Fraser index (8), RBC count and Green and King index (9).

After application of red blood cell indices, only those patients with possibility of β thalassemia trait were selected. 4 ml venous blood was drawn in EDTA vial from all these patients for HbA2 estimation by high performance liquid chromatography (HPLC) for confirmation. The system used for HPLC was BIO-RAD VARIANT Beta Thalassemia short program.

The collected data was analyzed statistically using Student 't' test and Mann Whitney test, wherever applicable respectively, to compare the hematological parameters of patients who were confirmed to have beta thalassemia trait & those who did not. The sensitivity & specificity of the various red blood cell indices used to screen for beta thalassemia trait was also calculated & the best index according to Youdens index found. (10)

RESULT

This study included 300 pregnant women with microcytosis (MCV<80) with or without anemia.

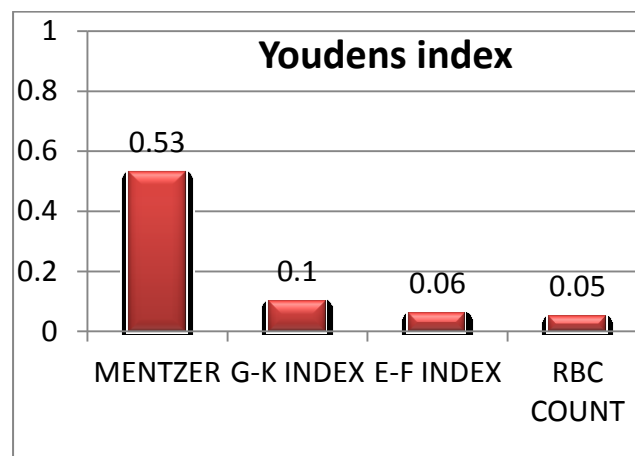
Table no 1 shows the comparison b/w the mean of CBC indices in the BTT and the non BTT group. which shows the statistically significant difference in MCV,MCH,RDW,PCV.

The table no .2 shows that 3 out of 5 patients (60%) suspected by Mentzer index were confirmed to be BTT. Only 2/29(6.89%) not suspected to be BTT turned out to be BTT by HPLC. Only 1/5 (i.e. 20%) patients suspected by England Fraser index were confirmed to be BTT. 4 out of 29 not suspected (13.7%), were confirmed as BTT by HPLC. 2 out of 12 patients (16.67%) suspected by RBC count were in fact BTT. 3 out of 22 not suspected (13.6%) turned out to be BTT by HPLC. Out of 31 patients suspected by Green King index 5 (16.13%) were confirmed as BTT. 3 patient not suspected remain as non BTT by HPLC .Those two patients who were not suspected by Mentzer index if we apply Green and king index and then reanalyzed along with Mentzer index we can further increases chances of positive results.

The table no .3 shows the comparison of the four red cell indices used for screening of BTT according to Youdens index. Mentzer index showed the low sensitivity of 60% but highest specificity of 93.10% .England Fraser index had a lowest Sensitivity of 20% but high Specificity

of 86.21%, while RBC count had Sn of 40% and specificity of 65.52%. The Green King index showed a highest sensitivity of 100% but a very low Sp of 10.34%.

GRAPH 1: COMPARISON OF THE VALIDITY OF THE FOUR RED CELL INDICES BY YOUDENS INDEX



This graph shows that Mentzer index has the best overall accuracy of +0.53 followed by Green King index(YI of+0.10),England & Fraser(YI of+0.06) and RBC count(YI of+0.05) So according to this study the Mentzer index is the best index to be used for screening patients of beta thalassemia trait

DISCUSSION

This study was undertaken to see the utility of erythrocyte indices for screening of beta thalassemia trait in pregnant women, as these indices are based on complete blood count reports which are routinely available and cost effective.

Out of the 300 cases of microcytosis, only 34 were suspected to have beta thalassemia trait after applying the four erythrocyte indices. Out of these 34 cases, 5 were confirmed to have beta thalassemia trait (BTT) by HPLC including 2 patient of borderline HbA2 of 3.4% . Remaining 29 out of 34 were reported to have normal

hemoglobin & has been referred to as non BTT group. Those pregnant women found beta thalassemia trait positive by HPLC her husband also investigated for beta thalassemia trait by HPLC so that we can prevent birth of a thalassemic child by taking action at appropriate time by termination of her pregnancy but BTT was not detected in any patient.

Positive family history and history of blood transfusion were not found in any patient in the BTT group in our study. Peripheral blood film of all patients showed microcytes, moderate hypochromia & mild anisocytosis.

This study showed a highly significant lower mean corpuscular volume (in fl) of 62.24 ± 4.36 in BTT group compared to 71.50 ± 5.09 in the non BTT group (Table 1). Amna A et al (11) reported an average MCV of 65.2 ± 5.75 . Ehsani et al (12) found average MCV to be 62.02 ± 4.57 in BTT group & 70.04 ± 7.94 in the IDA group. Zahid et al (13) found MCV was 66.82 ± 8.9 fl in BTT group.

The fact that chances of BTT increases as MCV value decreases, was shown in this study as in all other studies.

Mean MCH of the BTT group is lower than the non BTT group & this difference is significant (21.78 ± 3.27 vs. 23.29 ± 3.11 , $p < 0.01$) (Table 1). Ehsani et al (12) noted a MCH of 19.68 ± 1.53 in the BTT group & 21.30 ± 3.52 in the IDA group. Zahid et al (13) noted a MCH of 20.76 ± 4.69 in BTT group. A lower MCH is seen in beta thalassemia trait. The results of this study matched most other studies.

In this study there was significant difference in the red cell distribution width (RDW-CV) between the two groups. It was $17.68 \pm 2.33\%$ in BTT group & $15.26 \pm 1.28\%$ in the non BTT group (Table 1). RDW is normal in patients with beta thalassemia trait and increased in iron

deficiency anemia. This may be explained by coexisting iron deficiency anemia in some patients with beta thalassemia trait.

In this study there was significant difference in the Packed cell volume (PCV) between the two groups. PCV was 29.34 ± 3.23 in the BTT group & 34.55 ± 5.85 in the non BTT group (Table 1). This may be explained by coexisting iron deficiency anemia in some patients with beta thalassemia trait. There was no significant difference in the values of MCHC, platelet count, total leukocyte count.

Erythrocyte indices

1) Mentzer index: (TABLE 2,3)

Sensitivity (Sn) & specificity (Sp) of the index were 60% & 93.10% respectively, while the positive predictive value (PPV) & negative predictive value (NPV) were 60 & 93.10 respectively. Overall the validity of the index according to Youdens index was +0.53. Ehsani et al (12) reported a Sn & Sp of 95.5% & 94.6% respectively for Mentzer index with overall validity by Youdens index of +0.91. Ntaios et al (14) reported a Sn & Sp of 59.78% & 99% respectively with YI of +0.58.

Hence Mentzer index has a high validity in our study. The results of our study are comparable to other studies.

2) England Fraser index (TABLE 2,3)

Sensitivity (Sn) & specificity (Sp) of the index were 20% & 86% respectively, while the positive predictive value (PPV) & negative predictive value (NPV) were 13.79 & 86.21 respectively. Overall the validity of the index according to Youdens index was +0.06. Ehsani et al¹² reported a higher Sn & Sp of 69.5% & 99.2% respectively with overall validity by Youdens index of +0.68. Ntaios et al (14) reported a Sn & Sp of 64.07% & 99% respectively with YI of +0.63. Lower validity of this index obtained in our study.

3) RBC count (TABLE 2,3)

Sensitivity (Sn) & specificity (Sp) of the index were 40% & 65.52% respectively, while the positive predictive value(PPV) & negative predictive value (NPV) were 16.67 & 86.36 respectively. Overall the validity of the index according to Youdens index was +0.05 Ehsani et al(12) reported a higher Sn & Sp of 98.1% & 86.2% respectively with overall validity by Youdens index of +0.84. Ntaios et al(14) reported a Sn & Sp of 64.34% & 99% respectively with YI of +0.63.

As this study included pregnant womens in which iron deficiency anemia may be present which decreases HbA2 level this may explain the low validity of this index obtained in our study.

4) Green King index (TABLE 2,3)

Sensitivity (Sn) & specificity (Sp) of the index were 100% & 10.34% respectively, while the positive predictive value(PPV) & negative predictive value (NPV) were 16.13 & 100 respectively. Overall the validity of the index according to Youdens index was +0.10.

The low specificity of this index may be explained by the fact that anemia was not included as an inclusion criteria. Since hemoglobin is part of the denominator in Green King index, patients with normal hemoglobin levels were falsely suspected as BTT by this index.

According to the this study the Mentzer index had the best overall accuracy of +0.53 followed by Green King index(YI of+0.10),England & Fraser(YI of+0.06) and RBC count(YI of+0.05) . Hence in this study the Mentzer index is the best index to be used for screening patients of beta thalassemia trait.(TABLE 3) Ehsani et al(12) also reported Mentzer index (YI of +0.90) as the best discrimination index followed by RBC

count(+0.84) & England Fraser (+0.68). Ntaios et al(14) found Green King index (YI of +0.70) to be the best index in their study followed closely by England Fraser(+0.63), RBC count(+0.62) & Mentzer index(+0.58).

CONCLUSIONS

In our study the patients in the BTT group had significantly decreased mean corpuscular volume(MCV) , mean corpuscular hemoglobin(MCH) and packed cell volume(PCV) compared to those in non BTT group. There was significant difference in red cell distribution width (RDW) in the two groups.RDW was significantly high in the BTT group then non BTT group. This may be explained by coexisting iron deficiency anemia in some patients with beta thalassemia trait. Mentzer index was the best among the four discrimination indices used. The present study gives us an idea that the Mentzer index with CBC may be the simple, low cost, rapid and reliable screening test for thalassemia as a routine. Patient scrutinized by Green and king index then reanalyzed along with Mentzer index further increases chances of true positive results. Use of both indices reduce application of costly confirmatory test like HPLC.

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Table 1: Comparison of Mean of different CBC indices between BTT & Non BTT Groups

| Ttest | BTT | NBTT | P VALUE | Significance |
|-------|------------|------------|---------|--------------|
| HHb | 10.16±0.56 | 11.42±1.92 | >0.05 | NS |
| MCV | 62.24±4.36 | 71.5±5.09 | <.001 | S |
| MCH | 21.78±3.27 | 23.29±3.11 | <0.001 | S |
| RDW | 17.68±2.33 | 15.26±1.28 | <0.05 | S |
| TLC | 8.17±2.37 | 10.35±3.19 | >0.05 | NS |
| MCHC | 34.94±4.02 | 42.35±5.07 | >0.05 | NS |
| PLT | 2.25±0.5 | 2.25±0.69 | >0.05 | NS |
| PCV | 29.34±3.23 | 34.55±5.85 | <0.01 | S |

Table 2: Results Obtained Using Different Indexes

| | | TOTAL | HPLC | |
|-----------------------|----------|-----------|------|---------|
| | | | BTT | NON BTT |
| Mentzer | B | 5 | 3 | 2 |
| | I | 29 | 2 | 27 |
| ENGLAND FRASER | B | 5 | 1 | 4 |
| | I | 29 | 4 | 25 |
| RBC COUNT | B | 12 | 2 | 10 |
| | I | 22 | 3 | 19 |
| GREEN KING | B | 31 | 5 | 26 |
| INDEX | I | 3 | 0 | 3 |

Table 3: Comparison of the Results Obtained using the Four Indices to Detect BTT

| INDEX | SENSITIVITY | SPECIFICITY | PPV | NPV | YOUDENS INDEX |
|---------------------------|-------------|-------------|-------|-------|---------------|
| MENTZER | 60% | 93.10% | 60 | 93.1 | 0.53 |
| ENGLAND FRASER | 20% | 86.21% | 13.79 | 86.21 | 0.06 |
| RBC COUNT | 40% | 65.52% | 16.67 | 86.36 | 0.05 |
| GREEN KING INDEX | 100% | 10.34% | 16.13 | 100 | 0.1 |