DIAGNOSTIC SIGNIFICANCE OF RED CELL INDICES 
IN BETA-THALASSAEMIA TRAIT

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The purpose of this study was to evaluate the formulae for the diagnosis of beta-thalassemia trait cases in settings where electrophoresis is not available. The study included 50 cases of beta-thalassaemia trait already diagnosed by Hb. electrophoresis. CBC samples were analyzed on Sysmex K4500 and red cell indices were used to evaluate formulae for differentiating beta-thalassaemia trait from iron deficiency anaemia. The formula MCV/RBC and MCH/RBC identified 56% of the cases. Formula MCV - (5 × Hb)- RBC - 8.4 identified 54% of beta-thalassemia trait cases. The formula MCV × MCH identified 92% of cases. RBC indices given by electronic counters can be used to differentiate iron deficiency anaemia from beta-thalassaemia trait at least provisionally in areas where Hb. electrophoresis is not available.

Key words: Beta thalassaemia trait and red cell indices.

INTRODUCTION
Beta thalassaemia minor is the most common form of thalassaemia. It is common in Greeks, Turks, Cypriots, Italians and to a lesser extent the Indian subcontinent. However it can occur in subjects of any ethnic origin.1 Mostly the patients are diagnosed on routine blood examination and sometimes the disease manifests itself during stress such as pregnancy.2

The identification of β-thalassaemia minor is essential for two reasons. Firstly to differentiate it from iron deficiency since both present as microcytosis and hypochromia. Secondly for prevention of beta thalassaemia major by genetic counselling. Through genetic counselling birth rate of β-thalassaemia major can be reduced by as much as 90%.3

Haemoglobin electrophoresis is essential for definite diagnosis of β-thalassaemia trait cases. Normally Hb A2 is less than 3.2% but in β-thalassaemia it is more than 3.5%.4 In areas where modern equipments for diagnosis are not available, a simple morphologic criterion has been proposed. It is based on microcytic red cells, target cells and basophilic stippling on peripheral blood films. Red cell indices are used for initial screening of marriage cases in high risk areas.6 The red cell indices give more reliable diagnosis. In thalassaemia trait cases MCV and MCH are low while MCHC is normal. Red cell count is often more than 5.0×10^7. Measuring coefficient of variation by electronic counters is an excellent technique for rapidly distinguishing β-thalassaemia trait from iron deficiency anaemia. It is more than 14% in iron deficiency and less than 14% in β-thalassaemia trait.8 Several formulae have been proposed for differentiating iron deficiency from β-thalassaemia trait. Although these formulae may identify majority of uncomplicated cases they do not work well in children, during pregnancy or when there are complicating factors.1 The formula MCV-(5×Hb)-RBC-K, where K is a constant determined by calibrating the electronic counters, a positive value indicates iron deficiency and a negative value suggests β-thalassaemia trait9 In 1973 Metzner10 gave the formula MCV/RBC. According to this formula a value less than 13 indicates β-thalassemia trait. A similar formula MCH/RBC was also proposed.11 A value of over 4.4 indicates iron deficiency whereas a value under 4.4 indicates thalassaemia trait. MCV^2 × MCH/IOO is another formula in which a value less than 1530 indicates thalassaemia trait.12 Yet another method has been described.13 This method requires the availability of a newer generation of haematology analyzers which can give percentage of hypochromic red cells and percentage of microcytic red cells. The formula is discriminating beta-thalassaemia trait

index = RDW × MCV × % Hypochromic RBC + MCH 
100 × RBC × % microcytic RBC


According to them this formula is able to identify 100% of beta-thalassaemia trait cases.

This study was designed to evaluate reliability of some of the above mentioned formulae. The aim is to find some reliable means to provisionally diagnose thalassemia trait cases in settings where electrophoresis is not available.

**MATERIALS AND METHODS**

It was a retrospective study of 50 cases of β-thalassaemia trait from public sector hospitals of Lahore area. They were diagnosed on Hb. electrophoresis and for whom indices given by haematology analyzer were available. Haematology counter Sysmex K4500 was used for red cell indices. Data of these cases was scrutinized for the present study. However we are unable to analyze the formula given by Vicinanza because of non-availability of haematology analyzer which can give percentage of microcytic and hypochromic red cells.

**RESULTS AND OBSERVATIONS**

In the present study a total of 50 cases of β-thalassaemia trait diagnosed by Hb electrophoresis were included. Thirty (60%) of the subjects were females while 20 (40%) were males.

In all the cases the value of haemoglobin was low with a mean ± SD of 10.40 ± 1.77 gm/dl. Red cell count was 5.04 ± 0.97 × 10^12/L. Haematocrit value was 33.34 ± 5.4 L/L. The MCV was also low i.e 66.82 ± 8.94 fl. Similarly MCH was low i.e 20.76± 4.69 pg. The MCHC was normal i.e 31.08 ± 2.21 g/dl. Findings on Hb electrophoresis were characteristic. Hb A2 was in a range of 3.5 to 11.5%. The formulae previously mentioned were applied and the following results were found. MCV/RBC and MCH/RBC correctly identified 28 (56%) cases. The formula MCV-(5×Hb)-RBC-8.4 identified 27 (54%) cases of β-thalassaemia trait. Shine and Lal formula was relevant in maximum number i.e 46(92%) subjects of β-thalassaemia trait. All the four formula had a predictive value in 24 (48%) subjects and in 4 (8%) subjects three formulae had this value. In one case (2%) two formulae and in 14(28%) cases one formula had a positive predictive value for β-thalassaemia trait cases.

**DISCUSSION**

In the present study Hb, HCT, MCV and MCH were low and MCHC was normal which is comparable to studies conducted by other authors. Hb A2 is the important finding to diagnose β-thalassaemia trait cases which was high and very similar results were quoted by another group. Although the finding of a high HB A2 by Hb. electrophoresis is a gold standard for the diagnosis of β-thalassemia trait but it is not available at all places. Thus several attempts have been made to diagnose the condition by using red cell indices. In the present study MCV/RBC ratio was able to detect 28 (56%) cases of β-thalassaemia trait. The same ratio was studied by others. Other and concluded a predictive value of 87.4% for the correct identification of β-thalassaemia trait. MCH/RBC ratio was able to predict 28(56%) subjects having β-thalassaemia trait. The discriminant factor i.e MCV- (5 × Hb)-RBC-84 was able to predict 27 (54%) cases of β-thalassaemia trait. The same formula was applied predicted 417 out of 455 cases of β-thalassemia trait. In another study they were able to predict 398 out of 435 (91.5%) cases of β-thalassaemia trait. Shine and Lal formula MCV^× MCH was applied by England and Fraser, and it failed to distinguish iron deficiency from heterozygous β-thalassae-mia. In the present study it was able to correctly identify 46(92%) subjects having β-thalassaemia trait. In the present study we have selected only diagnosed cases of β-thalassaemia trait. These formulae are to be evaluated in cases having microcytic and hypochromic picture and there is a need for further evaluation by another study.

### Table 1: Formulae for distinguishing between p-thalassemia trait and iron deficiency anemia.

<table>
<thead>
<tr>
<th>Formula</th>
<th>Beta thalassaemia trait</th>
<th>Iron deficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCV / RBC</td>
<td>&lt;13</td>
<td>&gt;13.3</td>
</tr>
<tr>
<td>MCH / RBC</td>
<td>&lt;3.8</td>
<td>&gt;3.8</td>
</tr>
<tr>
<td>MCV - (Hb×5) -RBC-K</td>
<td>&lt;0</td>
<td>&gt;0</td>
</tr>
<tr>
<td>MCV^×MCH / 100</td>
<td>&lt;1530</td>
<td>&gt;1530</td>
</tr>
</tbody>
</table>

It was concluded that the facility for Hb electrophoresis is not available at many places in Pakistan. Red cell indices given by electronic counters can be reliably used to differentiate iron deficiency anaemia and β-thalassaemia trait. By applying the aforementioned formulae it is possible to identify majority of β-thalassaemia
Table 2: Average ± SD as obtained in the study.

<table>
<thead>
<tr>
<th>Hb</th>
<th>RBC</th>
<th>HCT</th>
<th>MCV</th>
<th>MCH</th>
<th>MCHC</th>
<th>HbA</th>
<th>HbA2</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.40</td>
<td>5.04</td>
<td>33.34</td>
<td>66.82</td>
<td>20.76</td>
<td>31.08</td>
<td>94.9</td>
<td>5.05</td>
</tr>
<tr>
<td>±7.77</td>
<td>±0.97</td>
<td>±5.40</td>
<td>±8.94</td>
<td>±4.69</td>
<td>±2.27</td>
<td>±7.27</td>
<td>±1.30</td>
</tr>
</tbody>
</table>

trait cases. Their accuracy is improved when read in conjunction with peripheral smears. Another study need to be carried out involving microcytic and hypochromic cases.

REFERENCES