Anemia is a public health problem that affects populations in both rich and poor countries and is widely prevalent amongst all the ages. Anemia resulting from lack of sufficient iron to synthesize hemoglobin is the most common hematological disease in infants and children. It has been estimated that 30% of the global population suffers from iron deficiency anemia (IDA), and most of those affected live in the developing countries. Recent NFHS–III (National Family Health Survey- III) surveys (2005–06) have shown that 70-85% (approx. 79.2%) of Indian young children have anemia. Thalassemia is one of the major autosomal recessive hereditary hemoglobinopathies prevalent in the world population, particularly in Mediterranean belt, Far-eastern and South East Asian countries. Thalassemias are a group of hemoglobinopathies caused by genetic mutations of the hemoglobin (Hb) genes, resulting in reduced production or total absence of one or more globin chains. Iron deficiency anemia is the most common microcytic hypochromic anemia worldwide. Anemia resulting from lack of sufficient iron to synthesize hemoglobin is the most common hematological disease in young children and women of reproductive age but it can be found in people of any age group. It has been estimated that 30% of the global population suffers from iron deficiency anemia (IDA) and most of those affected live in the developing countries like India. Iron deficiency anemia in adults is caused by loss of blood, while in childhood faulty diet is to blame. Currently, the detection of IDA is largely dependent upon quantification of biochemical markers like serum ferritin (SFr), serum transferrin (STr) and zinc protoporphyrin (ZnPp) which are not routinely available and affordable in developing countries due to high costs. Results & Observation. In the present study, Mentzer Index (MI) had a sensitivity of 71.91 and 84.62 % for IDA and BTT respectively. The specificity of MI for IDA and BTT was 84.62% and 71.91 % respectively. The PPV of MI for IDA and BTT was 94.12% and 46.81% respectively while the NPV of MI for IDA and BTT was 46.81 and 94.12% respectively. We can conclude that RBC count >4.69 million/mm3 and MCV < 62.9 fL favours the diagnosis of BTT while RBC count <4.69 million/mm3 and MCV >62.9 fL favours IDA. Conclusion: A limited specificity of RDW in diagnosis of IDA among children with microcytic hypochromic anemia suggests that further studies like serum ferritin, serum iron, serum TIBC, and hemoglobin studies used in a systemic manner are still necessary to make an accurate diagnosis of the cause of microcytosis. RDW with a good sensitivity can be used as good discriminative index between IDA and BTT. It can be used to differentiate microcytic anemias into IDA and BTT, where HPLC and iron studies (S. ferritin, S.iron, TIBC) cannot be done due to factors like unavailability or high cost.

KEYWORDS:
Microcytic hypochromic anemia, red cell distribution width

INTRODUCTION
Anemia is a public health problem that affects populations in both rich and poor countries and is widely prevalent amongst all the ages. Anemia resulting from lack of sufficient iron to synthesize hemoglobin is the most common hematological disease in infants and children. It has been estimated that 30% of the global population suffers from iron deficiency anemia (IDA), and most of those affected live in the developing countries. Recent NFHS–III (National Family Health Survey- III) surveys (2005–06) have shown that 70-85% (approx. 79.2%) of Indian young children have anemia. Thalassemia is one of the major autosomal recessive hereditary hemoglobinopathies prevalent in the world population, particularly in Mediterranean belt, Far-eastern and South East Asian countries. Thalassemias are a group of hemoglobinopathies caused by genetic mutations of the hemoglobin (Hb) genes, resulting in reduced production or total absence of one or more globin chains. Iron deficiency anemia is the most common hematological disease in young children and women of reproductive age but it can be found in people of any age-group. It has been estimated that 30% of the global population suffers from IDA and most of those affected live in the developing countries like India. Iron deficiency anemia in adults is caused by loss of blood, while in childhood faulty diet is to blame. BTT is an important differential diagnosis of IDA may closely mimic those that are present in iron deficiency anemia, the therapy is radically different. It is very important not to treat a patient with thalassemia with an iron supplement as this can lead to hemochromatosis (accumulation of iron in various organs, especially the liver). Thus reliable and efficient diagnostic ways to distinguish between thalassemia and iron restricted microcytic hypochromic anemia are desirable.

Studies have shown that iron deficiency causes delay in cognitive development and poor motor and sensory system functioning and that iron supplementation in early years may prevent these complications among children. Conversely, there is an evidence suggesting that routine iron treatment in non-iron deficient children may have adverse consequences for morbidity and infections. Currently, the detection of IDA is largely dependent upon quantification of biochemical markers like serum ferritin (Sfr), serum...
transferrin (STr) and zinc protoporphyrin (ZnP) which are not routinely available and affordable in developing countries due to high costs. A definitive differential diagnosis between TT and IDA is based on the result of HbA2 electrophoresis, serum iron levels, and a ferritin calculation.

Studies have shown that RDW, a measure of variations in the circulating RBCs, in addition to other hematological markers like mean corpuscular volume (MCV) and hemoglobin can be used as a differential diagnostic tool for identification of iron deficiency anemia.

Red cell distribution width (RDW) has been proposed to be a more sensitive indicator to establish the possible origin of microcytic hypochromic anemia. The RDW represents the coefficient of variation of the red blood cell volume distribution and can be considered an index of heterogeneity, the equivalent of anisocytosis observed in the peripheral blood smear. Various previous studies have debated the role of RDW in diagnosis of IDA with 38.3% of cases. Amongst the BTT and IDA group, the most common age group was 12-24 months (50% and 34.8% cases respectively). There was insignificant correlation between the prevalence in different age groups and the disease occurrence of BTT and IDA (p-value=0.449). Out of the total cases, 58.3% were females and 41.7% were males. Out of total BTT cases, 65.4% were females and 34.6% were males. Amongst all the IDA cases, 56.2% were females and 43.8% were males. However, no significant correlation between the age and the diagnosis was found (p-value=0.042). Almost all of the BTT cases (96.2%), presented with mild anemia with hemoglobin between 9-11g/dl (p-value=0.000, significant). While IDA patients presented with mild, moderate as well as severe degree of anemia. The mean value of hemoglobin in IDA group was statistically lower i.e. 7.68 g/dl (SD-2.468) as compared to BTT group with a mean Hb of 10.31 g/dl (SD-0.731) (p-value=0.000).

**RDW in IDA and BTT**

<table>
<thead>
<tr>
<th>RDW-CV (%)</th>
<th>Diagnosis</th>
<th>Total</th>
<th>Chi-square value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>11.6-14</td>
<td>BTT</td>
<td>0</td>
<td>2</td>
<td>2.2%</td>
</tr>
<tr>
<td>14-18</td>
<td>IDA</td>
<td>26</td>
<td>21</td>
<td>23.6%</td>
</tr>
<tr>
<td>More than 18</td>
<td>BTT</td>
<td>0</td>
<td>66</td>
<td>74.2%</td>
</tr>
<tr>
<td>Total</td>
<td>26</td>
<td>89</td>
<td>100.0%</td>
<td>115</td>
</tr>
</tbody>
</table>

Normal RDW-CV - 11.6 to 14%(12.8±1.2%) in all the cases of BTT, the RDW was only slightly elevated (value 14-18%), while in 74.2% of the IDA patients had RDW-CV values more than 18%, followed by 23.6% having RDW 14-18% while only a few cases (2.2%) showed RDW in the normal range (11.6-14%). But the p value obtained in this analysis was 0.090(>0.05), hence it was statistically insignificant. The mean value of RDW was 16.24% in BTT and 21.52% in IDA.

**Figure 1: ROC of RDW for the differentiation of BTT from IDA**

**Table 1:** Distribution of cases according to RDW in BTT and IDA

-Receiver operator curves (ROC) were constructed using RDW values of the children among two groups. The sensitivity, specificity, PPV and NPV were calculated using different cut-off of 15, 16 and 17% and the following results were obtained.

**RESULT**
The present study was conducted on a pediatric population (age group 1-5 years i.e. 12-60 months), with a hemoglobin <11 g/dL and an MCV of <80 fl. A total of 115 cases were analyzed. All these cases of microcytic hypochromic anemia were subgrouped as IDA and BTT on the basis of criteria already mentioned. Out of the total 115 cases, 22.6% were diagnosed as BTT and 77.4% were diagnosed as IDA. Out of the total 115 cases, the most common age group was 12-24 months with 38.3% of cases. Amongst the BTT and IDA group, the most common age group was 12-24 months (50% and 34.8% cases respectively). There was insignificant correlation between the prevalence in different age groups and the disease occurrence of BTT and IDA (p-value=0.449). Out of the total cases, 58.3% were females and 41.7% were males. Out of total BTT cases, 65.4% were females and 34.6% were males. Amongst all the IDA cases, 56.2% were females and 43.8% were males. However, no significant correlation between the age and the diagnosis was found (p-value=0.042). Almost all of the BTT cases (96.2%), presented with mild anemia with hemoglobin between 9-11g/dl (p-value=0.000, significant). While IDA patients presented with mild, moderate as well as severe degree of anemia. The mean value of hemoglobin in IDA group was statistically lower i.e. 7.68 g/dl (SD-2.468) as compared to BTT group with a mean Hb of 10.31 g/dl (SD-0.731) (p-value=0.000).
RDW cut-off value of 16% was the value with a best combination of sensitivity and specificity for IDA and BTT. At this RDW cut-off value of 16%, 94.38% of the IDA patients had RDW greater than 16% while only 1 patient had moderate anemia in BTT group had RDW cut-off value of 16% was the value with a best combination of sensitivity and specificity for IDA and BTT. At this RDW cut-off value of >16%, the sensitivity and specificity of RDW in differentiating IDA from BTT was found to be 87.64% and 57.69% respectively with a positive and negative predictive value of 87.64% and 57.69% respectively. The sensitivity & specificity of RDW to differentiate IDA from BTT according to different studies is as depicted in the table 5.

Table 2: Sensitivity, specificity, PPV, NPV for IDA and BTT according to different cut-off value of RDW-CV

<table>
<thead>
<tr>
<th>Cut-off value of RDW-CV (%)</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;15</td>
<td>IDA 94.38</td>
<td>BTT 7.69</td>
<td>44.38</td>
<td>28.57</td>
</tr>
<tr>
<td>&gt;16</td>
<td>IDA 87.64</td>
<td>BTT 57.69</td>
<td>37.64</td>
<td>76.64</td>
</tr>
<tr>
<td>&gt;17</td>
<td>IDA 77.53</td>
<td>BTT 40.77</td>
<td>30.77</td>
<td>93.24</td>
</tr>
</tbody>
</table>

RDW v/s degree of severity of anemia in IDA

Table 3: Mean value of RDW in mild, moderate and severe anemia in IDA

<table>
<thead>
<tr>
<th>Anemia vs RDW</th>
<th>No. of patients</th>
<th>Mean value of RDW</th>
<th>SD</th>
<th>Minimum</th>
<th>Maximum</th>
<th>P-VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-7 (severe)</td>
<td>32</td>
<td>25.03</td>
<td>5.704</td>
<td>16</td>
<td>38</td>
<td>0.000</td>
</tr>
<tr>
<td>7-9.0 (moderate)</td>
<td>24</td>
<td>19.80</td>
<td>3.901</td>
<td>13</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>9-11.0 (mild)</td>
<td>33</td>
<td>19.37</td>
<td>4.074</td>
<td>14</td>
<td>31</td>
<td></td>
</tr>
</tbody>
</table>

Amongst the IDA patients, the mean value of RDW was 19.37% in patients having mild anemia, 19.80% in patients with moderate anemia and 25.03% in severe anemia. Hence, the RDW increases with the severity of anemia in the IDA patients. The p value was 0.000. Hence it is highly significantly statistically.

RDW v/s degree of severity of anemia in BTT

Table 4: Mean value of RDW in mild, moderate and severe anemia in BTT

<table>
<thead>
<tr>
<th>Anemia vs RDW</th>
<th>No. of patients</th>
<th>Mean value of RDW</th>
<th>SD</th>
<th>Minimum</th>
<th>Maximum</th>
<th>P-VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>7-9.0</td>
<td>1</td>
<td>18.00</td>
<td>1.003</td>
<td>15</td>
<td>18</td>
<td>0.089</td>
</tr>
<tr>
<td>9-11.0</td>
<td>25</td>
<td>16.17</td>
<td>1.003</td>
<td>15</td>
<td>18</td>
<td></td>
</tr>
</tbody>
</table>

Most of the patients in BTT group presented with mild anemia, showed a mean RDW value of 16.17% while only 1 patient had moderate anemia in BTT group had RDW value of 18.00%.

Though this increase in the value of RDW according to the severity of anemia was statistically insignificant in BTT group.

DISCUSSION:

In our study, almost all of the BTT cases (96.2%), presented with mild anemia with hemoglobin between 9-11 g/dL. (p-value 0.000, significant). While IDA patients presented with mild, moderate as well as severe degree of anemia.

The findings and recommendations of western studies on RDW in iron deficiency anemia and beta-thalassemia trait may not be entirely applicable in a country like ours with a much higher percentage of IDA. Children with BTT, who have associated IDA, are likely to have a consequent high RDW and stand to be labeled as iron deficient initially. Careful follow up after adequate iron therapy following which the RDW may come back to normal but the low indices persist, may be required to then further investigate them for the appropriate heterozygous hemoglobinopathy.

It is well known that in certain conditions of concomitant diseases (for example, iron deficiency anemia and chronic disease anemia), even the results of gold standard tests may suffer the interference of the intercurrent disease, making diagnosis more difficult. Therefore, the results of our study indicate a limited usefulness of RDW as an auxiliary parameter for differentiation of these types of anemia, because a better discrimination between both nosological entities by RDW would depend on the absence of other disorders favoring microcytosis. Hence, in the absence of intercurrent disorders, it may be an important tool for differential diagnosis between iron deficiency anemia and thalassemia minor (alpha and beta), and it must be valued as an auxiliary laboratory parameter for this purpose.

A limited specificity of RDW in diagnosis of IDA and limited sensitivity in diagnosis of BTT among children with microcytic hypochromic children suggests that further studies like serum ferritin, serum iron, serum TRB, bone marrow biopsy and hemoglobin studies used in a systemic manner are still necessary to make an accurate
diagnosis of the cause of microcytosis.

CONCLUSION:
Cut-off value of 16% offered the best combination of sensitivity and specificity for IDA and BTT. At this RDW cut-off value of >16%, the sensitivity and specificity of RDW in differentiating IDA from BTT was found to be 87.64% and 57.69% respectively with a positive and negative predictive value of 87.64% and 57.69% respectively. A limited specificity of RDW in diagnosis of IDA among children with microcytic hypochromic anemia suggests that further studies like serum ferritin, serum iron, serum TIBC, and hemoglobin studies used in a systemic manner are still necessary to make an accurate diagnosis of the cause of microcytosis. RDW with a good sensitivity can be used as a good discriminative index between IDA and BTT. It can be used to differentiate microcytic anemias into IDA and BTT, where HPLC and iron studies (S. ferritin, S.iron, TIBC) cannot be done due to factors like unavailability or high cost. Though hemoglobin studies and iron profile still remain the gold standard. However, preliminary screening of microcytic hypochromic anemia cases on routine complete blood count report with the help of RDW can avoid unnecessary iron therapy in children with suspected BTT as it may cause iron overload and systemic damage.

CONFLICT OF INTEREST
The authors had no conflict of interest to declare in relation to this article.

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REFERENCES