Original Research Article

Coexistence of iron deficiency and thalassemia trait: a study in antenatal females

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ABSTRACT

Background: Thalassemia is most common genetic disorder worldwide and about 7% of world population is carrier. The prevalence of Beta thalassemia trait (BTT) is 3.5–10% in India. The National Family Health Survey (NFHS-3) of 2011 reveals the prevalence of iron deficiency anemia (IDA) as 70–80% in children, 70% in pregnant women, and 24% in adult men. As both of them are close differential diagnosis and both can coexist together, this study aims to detect hemoglobinopathies in pregnant women and quantify the effect of iron deficiency on HbA2 levels in order to improve the detection of β thalassemia trait with and without iron deficiency.

Methods: Hb, RBC indices, and peripheral smears of 90 pregnant females with microcytic hypochromic blood picture were studied. Serum ferritin and HPLC (High Performance Liquid Chromatography) were performed. The results were analysed statistically by using SPSS version 16.0.

Results: 93.3% patients had HbA2 <2%, 4.44% had >4.0% which characterise BTT and remaining 2.22% had between 3.0%-4.0%. HbA2 <2.0% may be seen in IDA, ATT, HbH disease and Delta thalassemia. 91.11% had reduced serum ferritin and 2.22% had normal ferritin levels.

Conclusions: This study reveals that there is frequent occurrence of iron deficiency anaemia in patients with thalassemia traits. This can substantially invalidate the diagnosis of the latter. Hence, iron deficiency should be identified and rectified in patients with suspicion of thalassemia trait.

Keywords: Beta thalassemia trait, Iron deficiency anemia, Alpha thalassemia trait, Hemoglobin A2

INTRODUCTION

Thalassemia is one of the most common single gene disorder representing a major health burden in India and in the world. They are a group of autosomal recessive disorders in which there is inhibition in production of α or β globin chains of haemoglobin resulting in varying levels of anaemia. 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.

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.

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.
Various screening parameters are available which include peripheral blood smear examination, red cell osmotic fragility test, free red cell porphyrins and red cell indices which can then be confirmed by HPLC and mutational analysis.\(^5\)

Complete blood counts including red cell indices given by automated haematology analyser are major contributors for screening and 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.

Due importance to the peripheral smear and red cell indices in complete blood count should be given, so that suspicion of thalassemia is not missed. Different studies have shown different indices to be superior in predicting beta thalassemia trait.\(^6\)

The confirmation of these cases further rest on High performance liquid chromatography (HPLC) and/or mutational analysis.

HbA2 determination plays a key role in detection of β-thalassemia because small increase in this fraction is the most important marker of β-thalassemia heterozygous carriers.\(^7\)

Reduced production of β-globin, with relative excess of α-globin chains, and “compensatory” increase in δ-globin synthesis, favor the formation of αδ dimers and assembly of HbA2 tetramers. Low HbA2 values, in most instances are the result of either reduced synthesis of the δ-globin chain, or posttranslational modifications in the assembly of the HbA2 tetramer due to a reduction in the synthesis of α-globin chains.\(^8\)

Few studies report that iron deficiency (ID) is a potential source of diagnostic interference in tests for HbA2 determination that may give false positive or negative results.\(^9\)

In fact, intracellular lack of iron reduces α-globin chain synthesis relative to that of non-α globin chains; when the supply of β-globin chains is limited, β-globin chains compete more effectively for α-globin chains than δ-globin chains, resulting in reduced levels of Hb A2.\(^9\)

However, according to some studies β-thalassemic trait does not confer an advantage in maintaining iron balance, and that HbA2 is not significantly lowered in the presence of ID.\(^10\)

So, this study aims to detect hemoglobinopathies in pregnant women attending antenatal clinic on the basis of peripheral smear and red blood cell indices and its confirmation by high performance liquid chromatography. Also, we quantify the effect of iron deficiency on HbA2 levels in order to improve the detection of β thalassemia trait with and without iron deficiency.

### METHODS

Present study was conducted at Pathology department of a tertiary care hospital from July 2016 to December 2016. The subjects included all pregnant females (300) in the third trimester of pregnancy, attending regular antenatal clinic. Of these subjects, 90 pregnant females with microcytic hypochromic blood picture were included in the study group for which consent was taken from all the participants.

Five ml of venous blood was taken, out of which three ml was taken in EDTA vial and remaining two ml blood was collected in plain vial. The haematological parameters, haemoglobin along with RBC indices (PCV, MCV, MCH and MCHC) were analysed by three-part haematology analyser. Two peripheral blood smears were also made and stained by Leishman’s stain for each case. Serum ferritin and HPLC (High Performance Liquid Chromatography) was done on all the samples with microcytic hypochromic blood picture. The cationic exchange column chromatography enables quantitative determination of HbA2, HbF and abnormal haemoglobins. 10 μl haemolysis mixture prepared by diluting 5 μl of whole blood with 1 ml haemolysis reagent was injected into HPLC system. A flow rate of 1.5 ml/min, with an analytical run time of 6.5 min as recommended in kit protocol, was set. A cut off Hb A2 level of ≥3.5% was used for diagnosing thalassaemia trait.

Serum ferritin was done by the principle of microplate immunoenzymometric assay. Serum ferritin level of <10µg/dl was taken as cut off for diagnosis of iron deficiency anaemia. The results were analysed statistically by using SPSS version 16.0. (p < 0.05 was considered as statistically significant).

### RESULTS

**Beta-thalassemia trait (BTT) with iron deficiency anaemia (IDA)**

Out of 90 pregnant females, two (2.22%) females had thalassemia with iron deficiency anaemia There was a statistically significant difference in the mean values of Hb of BTT with coexistent IDA when compared with group of IDA (p=0.03). The mean HbA2 was significantly (p=0.0001) higher in BTT with IDA group as compared with IDA. The mean red blood cell (RBC) count, MCV, MCH, MCHC, RDW, HbA and HbF were not statistically significant (Table 1).

**Beta-thalassemia trait (BTT) without iron deficiency anaemia (IDA)**

Four (4.44%) females had BTT (without IDA). The mean red blood cell (RBC) count was significantly higher (p=0.04) in BTT group as compared to IDA group. The mean HbA2 was significantly (p=0.0001) higher in BTT
group as compared with the IDA group. There was statistically significant difference in HbA and HbF levels (p<0.0001).

The mean Hb, MCV, MCH, MCHC and RDW were not statistically significant in IDA and BTT subjects.

Table 1: Correlation between mean and standard deviation of various haematological parameters.

<table>
<thead>
<tr>
<th>Hematological parameters</th>
<th>Iron deficiency Iron deficiency anaemia (IDA) N=82 (mean±SD)</th>
<th>α-thalassemia trait (ATT) N=2 (mean±SD)</th>
<th>β-thalassemia trait (BTT) N=4 (mean±SD)</th>
<th>β-thalassemia Trait (BTT) with co-existent IDA N=2 (mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbH</td>
<td>8.21±1.69</td>
<td>7.88±1.27</td>
<td>9.05±0.05</td>
<td>10.8±1.97</td>
</tr>
<tr>
<td>Red blood cell count (RBC Count)</td>
<td>3.53±0.71</td>
<td>3.65±0.35</td>
<td>4.98±5.77</td>
<td>4.55±0.84</td>
</tr>
<tr>
<td>Mean corpuscular volume (MCV)</td>
<td>66.72±4.93</td>
<td>68.95±1.48</td>
<td>63.37±2.96</td>
<td>65.85±6.83</td>
</tr>
<tr>
<td>Mean corpuscular hemoglobin (MCH)</td>
<td>24.94±6.28</td>
<td>21.4±1.41</td>
<td>18.65±2.13</td>
<td>22.38±5.21</td>
</tr>
<tr>
<td>Mean corpuscular hemoglobin concentration (MCHC)</td>
<td>28.53±3.22</td>
<td>28.35±1.48</td>
<td>28.00±00</td>
<td>27.65±3.04</td>
</tr>
<tr>
<td>Red cell width (RDW)</td>
<td>19.41±5.02</td>
<td>17±0.84</td>
<td>17.35±0.51</td>
<td>16.4±2.72</td>
</tr>
<tr>
<td>HbA</td>
<td>96.67±0.56</td>
<td>97.45±0.07</td>
<td>92.35±1.55</td>
<td>96±0.21</td>
</tr>
<tr>
<td>HbA2</td>
<td>2.43±0.46</td>
<td>1.75±0.07</td>
<td>5.99±0.57</td>
<td>4.0±1.53</td>
</tr>
<tr>
<td>HbF</td>
<td>0.89±0.37</td>
<td>0.8±0</td>
<td>1.75±0.98</td>
<td>0.8±0</td>
</tr>
</tbody>
</table>

**Alpha-thalassemia trait (ATT) without iron deficiency anaemia (IDA)**

Two (2.22%) females had ATT without IDA. The mean HbA2 was in concordance in the ATT group when compared with the IDA group. The mean Hb, mean red blood cell (RBC) count MCV, MCH, MCHC, RDW, HbA and HbF were also not significantly different in IDA and ATT subjects.

93.3% females had HbA2 <2%, 4.44% patients had HbA2 > 4.0% which is characteristic of BTT and 2.22% had HbA2 levels between 3.0%-4.0%. 93.33% of our subjects had HbA2 levels. Those with HbA2 <2%, 91.11% had serum ferritin <10µg/dl and 2.22% had normal ferritin levels.

**DISCUSSION**

Among all the various population groups studied type II an Iron deficiency anaemia and thalassaemia syndromes, especially beta thalassaemia trait (BTT), are the two most frequent microcytic hypochromic anaemias highly prevalent in countries like India.\(^{1,12}\) The National Family Health Survey (NFHS-3) of 2011 reveals the prevalence of anaemia as 70-80% in children, 70% in pregnant women, and 24% in adult men. The prevalence of BTT has been cited as 3.5-10% in India.\(^{13,14}\) We classified the pregnant females in three different groups. Group I had BTT co-existent with IDA. In this group, we observed that there was a statistically significant difference in the mean values of Hb when compared with group of iron deficiency anaemia without thalassemia (p<0.03). The mean HbA2 was significantly (p<0.0001) higher in the thalassemia with anaemia group as compared with the anemic without thalassemia group. The mean red blood cell (RBC) count, MCV, MCH, MCHC, RDW, HbA and HbF were not significantly different in anemic and non-anemic thalassemic subjects. Some authors have reported that iron deficiency (ID) is a potential source of diagnostic interference in tests for HbA2 determination that may give false-positive or negative results.\(^9\) In fact, intracellular lack of iron reduces α-globin chain synthesis relative to that of non-α globin chains; when the supply of β-globin chains is limited, β-globin chains compete more effectively for α-globin chains than δ-globin chains, resulting in reduced levels of Hb A2.\(^8\) Few studies report that the β-thalassemic trait does not confer an advantage in maintaining iron balance, and that HbA2 is not significantly lowered in the presence of IDA which was in concordance with present study.\(^{15}\)

Group II included BTT (without co-existent IDA), RBC count was higher (p<0.04) in BTT group as compared to IDA. HbA2 was (p<0.0001) higher in the thalassaemic than IDA. There was statistically significant difference in the HbA and HbF levels (p<0.0001) while Hb, MCV, MCH, MCHC and RDW showed no significant difference. Elevated HbA2 level (≥3.5%) is an established screening test for BTT.\(^{16}\) However, few conflicting reports question the reliability of this test to screen BTT in the presence of iron deficiency.
Group III included ATT without IDA. HbA2 values were found similar in both ATT and IDA groups. Hb, RBC count, MCV, MCH, MCHC, RDW, HbA and HbF were not significantly different in IDA and ATT subjects. ATT is a close differential diagnosis of IDA even in the second level of diagnosis, as both have HbA2 <2%. Measurements of serum ferritin can provide laboratory evidence to exclude iron deficiency and ATT can be confirmed by mutational analysis.

Failure to exclude iron deficiency anemia in a patient with an alpha thalassemia syndrome may lead to continuation of supplemental iron therapy for an extended period, and the resulting iron overload may lead to secondary hemochromatosis. If iron overload continues longer than 1-2 years, it can lead to damage in multiple organs, including cardiac, hepatic, and endocrine dysfunction.

BTT and ATT patients with and without iron deficiency were compared to determine the effect of iron deficiency on the expression of HbA2. We observed that 4.44% patients had HbA2 >4.00% which is characteristic of BTT. 2.22% had HbA2 between 3.0%–4.0% which are considered intermediate and may require a more sensitive method for accurate determination. 93.33% of our subjects had HbA2 levels less than 2.0% which may be seen in IDA, ATT, HbH disease and Delta thalassemia. 91.11% were seen to have reduced serum ferritin and 2.22% had normal ferritin levels. Hence, we observed 91.11% had IDA, emphasizing that when HbA2 levels are less than 2.0%, IDA should always be ruled out. The remaining 2.22% with normal ferritin levels and with HbA2 levels <2% were referred for mutational analysis.

Current results show that the presence of iron deficiency did not preclude the detection of classical β carrier in our population. There could be some problems in the presence of silent β mutation or α gene triplication with ID, because HbA2 shows almost normal levels.

Apart from few limitations of this study, small sample size and unavailability of mutational analysis, present study highlights the prevalence of beta thalassemia in pregnant females. The effect of iron deficiency on HbA2 levels was studied to improve the detection of beta thalassemia trait with or without iron deficiency.

It is the need of the hour is to have a national policy on thalassemia for creating awareness about the disease and thus preventing its spread. The most effective approach to reduce the burden of the society and reduce the disease incidence is implementation of a carrier screening program, offering genetic counseling, prenatal diagnosis, and selective termination of affected foetuses.

**CONCLUSION**

Universal Screening for thalassemia carriers should be offered to all pregnant women attending antenatal clinic. Ideally, it should be done preconceptionally or as early as possible in the pregnancy. Implementation of a simple carrier screening in pregnancy is feasible in India. Along with screening of BTT in pregnancy, differentiation of BTT with or without iron deficiency anemia are essential because of the presence of microcytic hypochromic anemia in these cases.

The phenotypic expression of thalassemia trait is very diverse, and it has been thought that iron absorption in these individuals is increased. But this observation does not always exclude iron deficiency to have a contribution to anemia among the thalassemia trait. Clinical iron deficiency anemia may frequently co-exist with beta thalassemia trait and iron supplementation is invariably needed in deficient subjects. So, co-existing iron deficiency should always come under consideration among anemic individuals with thalassemia trait in the context of low socio-economic condition of India.

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**Ethical approval:** The study was approved by the Institutional Ethics Committee

**REFERENCES**
