Coexisting iron deficiency anemia and thalassemia trait


Abstract

The proportion of hemoglobin types may be affected by both the genetic and enviromental factors. Hypochromic and microcytic red cell morphology is the most commonly encountered abnormality in a hematology laboratory. This study was carried out on 200 cases of mild to moderate anemia with hypochromic microcytic blood picture and their iron status and hemoglobin A2 and Hb-E levels were determind to identify the presence of coexisting iron deficiency anemia and thalassemia carriers also called traits (both beta thalassemia trait and Hb-E trait). The full blood count was performed by hematology auto-analyzer, intial carrier detection of thalassemia was done by NESTROF (naked eye single tube red cell osmotic fragility) and subsequently confirmed by hemoglobin electrophoresis which was taken as gold standard; serum ferritin by radioimmunoassay (RIA) and Hemoglobin A2 and Hemoglobin E levels were estimated by Agarose gel Hb electrophoresis at an alkaline pH (8.6). The importance of the study of Peripheral Blood film (PBF) with red cell indices, serum ferritin and Hb electrophoresis are prerequisite for proper diagnosis of coexisting iron deficiency anemia (IDA) and thalassemia trait (and most of them are either beta thalassemia trait or hemoglobin-E trait).

Follow up examination with oral ferrous sulphate therapy revealed a definite restoration of hemoglobin A2, and Hb-E in corresponding study subjects toward the ratios of observed in subjects with non-iron deficiency. The response to a short course of oral iron therapy should therefore be carefully monitored, and the possibility of thalassemia traits (or carriers) as well as non-compliance with treatment should be considered and enthusiastic screening for hypochromic microcytic anemia is of prime importance to avoid the unnecessary use of iron supplements.

Introduction

A normal adult human has at least three types of hemoglobin, namely, A, A2, and F with the molecular formulas of a2 b2, a2d2, and a2y2 respectively. Hemoglobin A2, constitutes approximately 3.0% and Hemoglobin F less than 1% in the normal person and the rest is hemoglobin A1. These proportions are remarkably constant among the normal people, illustrating precise quantitative control of hemoglobin synthesis. However, the proportions of the hemoglobin types may be altered by several reasons.

It has been estimated that about 20% of the world population are iron deficient and iron
Deficiency anemia is the most common type of anemia throughout the world and dietary deficiency is the commonest cause. Iron is an essential element in humans, being the central iron in heme, the non-protein constituent of hemoglobin. Hemoglobin is responsible for the transport and delivery of oxygen from the lungs to the tissues and iron deficiency causes failure of heme synthesis.

On the other hand, thalassemia is the commonest inherited gene disorder prevalent worldwide. Bangladesh lies in the thalassemia belt and Beta-thalassemia is common here. World Health Organization (WHO) estimates that at least 7.0% of the world populations are carriers of different inherited disorders of Hemoglobin. It is predicted that when the world population finally stabilizes, at least 8.0% of the population will be carrier or trait. The world population of carriers of beta thalassemia trait is reported to be more than 100 millions worldwide and about 100,000 children with thalassemia major are born each year. Abnormal hemoglobin, called hemoglobin-E, which is quite common in Bangladesh, has also a worldwide carrier of about 53 millions. In Bangladesh no definite data regarding carrier status of hereditary hemoglobin disorder exist. No screening programme had ever been taken in any population group. A conservative World Health Organization (WHO) report estimates that about 3.0% of populations are carriers of Beta thalassemia and 4.0% are carriers of Hb-E in Bangladesh, which means that there are about 3.6 millions carriers of beta thalassemia and 4.8 millions are carriers of Hb-E and affected birth per thousand of Beta thalassemia is 0.106 & 0.300 of Hb-E/ Beta thalassemia. It is presumed that approximately six thousands thalassemic children are born each year in Bangladesh.

Materials and methods
The study was conducted in the Dept. of Pathology, Bangladesh Institute of Child Health and Dhaka Shishu Hospital, Sher-e Bangla Nagar, Dhaka from January 2000 to November 2001 to identify the cause of hypochromic microcytic blood picture and find out the coexisting of Iron Deficiency Anemia (IDA) and thalassemia trait. A total of 200 patients with mild to moderate anemia from various reasons were included in this study. The diagnosis of iron deficiency anemia was based on the clinical findings, the presence of hypochromic microcytic blood picture, low serum ferritin levels (<10 ng/ml). These patients usually came in with chronic anemia, many having sore tongues, angular stomatitis, cheliosis and koilonychia. After initial examinations, they were given oral ferrous sulphate daily, and when, possible, follow-up examinations were performed after 12 weeks of iron supplements. A few patients, who fit the clinical and red cell morphologic criteria but lacked the serum ferritin one and who responded well to iron therapy, were also included. A reduction in the size of the red cells (microcytosis) was defined volume of<74 fl based on the lower limit for the mean cell reference range (mean+2 SD) defined by Issaacs et al. If the MCV was <74 fl and the percentage of HbA2 was greater than 3.5% beta thalassemia trait was diagnosed. Similary, if the percentage of Hb-E was between 20-35% Hb-E trait was considered. The patients were offered oral iron supplements (2-3 mg/kg elemental iron as ferrous sulphate) for 12 weeks, after which the blood test was repeated. The response to oral iron therapy was considered to confirm iron deficient erythropoiesis if the MCV is increased by at least 5 fl or >74 fl.

Standard hematological techniques were employed and the study subjects were routinely tested for the followings: Red cell count, Hb (g/dl), Red cell indices (MCV, MCH & MCHC) by hematology autoanalyzer, hemoglobin phenotype (HbA2 and Hb-E) was identified by agarose gel electrophoresis, serum ferritin (RIA) and the naked Eye single tube red cell osmotic fragility test (NESTROF). NESTROF was carried out as advocated by mehat et al and kattamis et al.
Results
The age and sex distribution of the study cases are presented in Table I. Of the total 200 cases, 113 (56.5%) were males and 87 (43.5%) were females with a male to female ratio of 1.3:1. The age of the patients ranged from 5-45 years.

Of the 200 anemic patients (diagnosis was based on the clinical findings, the presence of hypochromic-microcytic blood picture, low serum ferritin<10 ng/ml level and phenotypic pattern of Hb electrophoresis), 177 were normal Hb electrophoresis with evidence of iron deficiency (IDA), 08 were b-thalassemia trait and 15 were Hemoglobin-E trait in association with IDA (Table II). These cases were re-evaluated before giving oral iron supplement (Table-II). After 12 weeks of iron supplements, results are re-evaluated and showed that out of 200 patients, 174 patients, are iron deficient usually for anemia of chronic disorders or due to hookworm infestations, 11 patients were beta-thalassemia trait and 15 patients were Hb-E trait in association with IDA (Table-III). These cases are reevaluated with iron supplement. However, the rates of increment of the hemoglobin, hematocrit, HbA2 and Hb-E during the iron supplement were irregular.

Table - I : Age & sex distribution of study cases n=200Female

<table>
<thead>
<tr>
<th>Age group in year</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-9</td>
<td>34</td>
<td>26</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>(17%)</td>
<td>(13%)</td>
<td>30%</td>
</tr>
<tr>
<td>10-14</td>
<td>43</td>
<td>31</td>
<td>74</td>
</tr>
<tr>
<td></td>
<td>(21.5%)</td>
<td>(15.5%)</td>
<td>37%</td>
</tr>
<tr>
<td>15-45</td>
<td>36</td>
<td>30</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td>(18%)</td>
<td>(18%)</td>
<td>33%</td>
</tr>
<tr>
<td>Total</td>
<td>113</td>
<td>87</td>
<td>200</td>
</tr>
<tr>
<td></td>
<td>(56.5%)</td>
<td>(43.5%)</td>
<td>100%</td>
</tr>
</tbody>
</table>

Table - II : Summery of the tests parameters in the study subjects before iron therapy (mean values)

<table>
<thead>
<tr>
<th>No. of the patients (n=200)</th>
<th>Hb (gm/dl)</th>
<th>Red cell count X1012/L</th>
<th>Hct (%)</th>
<th>MCV (fl)</th>
<th>MCH (pg)</th>
<th>S. ferritin (ng/ml)</th>
<th>Pattern of Hb electrophoresis</th>
</tr>
</thead>
<tbody>
<tr>
<td>IDA (n=177)</td>
<td>8.40</td>
<td>4.30</td>
<td>27.45</td>
<td>66</td>
<td>8.25</td>
<td>Hb A 96.30</td>
<td></td>
</tr>
<tr>
<td>Beta thalassemia trait with IDA (n=11)</td>
<td>11.45</td>
<td>5.40</td>
<td>35</td>
<td>74</td>
<td>63</td>
<td>Hb A 93.40</td>
<td></td>
</tr>
<tr>
<td>Hemoglobin-E trait with IDA (n=15)</td>
<td>11.85</td>
<td>5.10</td>
<td>38</td>
<td>75</td>
<td>66</td>
<td>Hb A 73.30</td>
<td></td>
</tr>
</tbody>
</table>

Eight patients were diagnosed as b-thalassemia trait with IDA during their initial screening. In these patients the hemoglobin A2 values subsequent to iron therapy were elevated to the range usually observed in b-thalassemia trait; the hypochromicity and microcytosis of the erythrocytes were also marked than in the iron deficient individuals who were homozygous for hemoglobin A. Another three patients, who were initially diagnosed as IDA on the basis of clinical findings, peripheral blood film, serum ferritin and HbA2, subsequent electrophoresis after 3 months of oral iron therapy shows that they were b-thalassemia trait (HbA2 >3.5%). In each of these patients the amount of hemoglobin A2. increase as did the Hematocrit and Hb concentration following iron therapy.

Fifteen patients were diagnosed as Hb-E trait with IDA during their initial screening. The average hematocrit of 15 patients with hemoglobin E was 17.5. percent. The concentration of Hb-E varied between 17-27%, averaging 20.56% (in contrast to their normal...
values of 20-35%, who were not iron deficient). After 12 weeks of oral iron therapy, with the exception of one case, there was an increase in the amount of Hb-E with the hematocrit and hemoglobin concentrations, reaching the normal range in most cases. Other hematological parameters like MCV and MCH were also performed showing a definite restoration to slight extent but does not reach the normal reference values.

The study result was also interpreted with red cell counts and red cell indices (MCV, MCH and MCHC). Low MCV (<74 fl) & MCH (<27pg) with a relatively high red cell counts are associated with either beta-thalassemia trait or Hb-E trait and low MCV, MCH and low red cell counts are usually associated with iron deficiency Anemia (IDA).

Discussion
The purpose of this study was to evaluate the existence of concomitant IDA and thalassemia trait (both beta-thalassemia trait & Hb-E trait) in a hypochromic microcytic blood picture which is a major problem in the correct diagnosis of Beta thalassemia trait & Hemoglobin-E trait and IDA.

In coexisting IDA with thalassemia trait, there is drop of hemoglobin A2 & Hb-E3,5,11. Due to fall of hemoglobin A2 it creates a much diagnostic problems because we can say as beta-thalassaemia trait only when HbA2 is more than 3.5% which frequently arises in association with coexisting b-thalassaemia trait and iron deficiency anaemia. HbE also decreases but it does not create much diagnostic problems because Hb-E level 20-35% is the diagnostic hallmark for hemoglobin-E trait11. However, in many patients with beta-thalassemia trait and iron deficiency, the Hb A2 will still be raised or normal. It is important to note that in beta-thalassemia trait the concentration of hemoglobin A2 which is usually elevated, may be significantly reduced in iron deficiency and that the, diagnosis of this conditions is not possible on the basis of Hb electrophoresis. This is of particular importance when a study is conducted in an area where iron deficiency is also prevalent. No significant alteration in the amount of the alkali-resistant Hemoglobin was observed in iron deficiency. This hemoglobin is normally present in minute amounts, <1%11. The altered proportions of hemoglobin A2 in iron deficiency anemia may not have a major physiological effect, since its amount is so low in any case. But hemoglobin E constitutes 20-35% of the hemoglobin in heterozygotes and almost 100% in homozygotes11,12. Since intraerythrocytic hemoglobin E has lower oxygen affinity than hemoglobin A in similar condition, it will, therefore, release oxygen to the tissues more readily than the normal hemoglobin and would then be particularly useful in anaemic state. The reduction of the hemoglobin E/A ratio in iron deficiency anemia thus seems to deprive its carrier of the expected benefit. Under the stress of iron deficiency the possession of hemoglobin E would appear to be a further disadvantage, if it is subject to a more drastic reduction than hemoglobin A, carriers of hemoglobins E would be more anemic than a normal persons being equally iron deficient. However, this will not be the case if the reason for the decreased hemoglobin E/A ratio is a partial switch-over of hemoglobin synthesis from hemoglobin E to A11.

Possible mechanisms responsible for alterations in hemoglobin ratios under iron deficiency should now be briefly considered : First, in iron deficiency anemia there is increased red cell destruction. If there is an unequal distribution of hemoglobin types among the red cell populations, differential destruction of the erythrocytes will lead to alteration of the hemoglobin ratios11. There is not yet evidence that haemoglobins A, A2 & E are contained in different erythrocytes. Second, if different peptide chains compete for heme moieties which are adequate for all people in a normal condition, then in iron deficiency which causes a heme deficit, the usual proportion of the haemoglobin types will be altered in favor of the one competing better. Third, iron may directly affect the rate of
globin synthesis\(^{11}\). Speculatively, this may happen at any of the multiplicity of steps along the pathway of protein synthesis. Recent in vitro observations have demonstrated the role of iron in globin synthesis and polysome function. The lack of iron in the incubation mixture causes retardation in the rate of globin synthesis and desegregation of polysomes. If explanation for the altered expressivity of hemoglobin types is to be on the basis, it is necessary to hypothesize that polysomes bearing different messenger RNAs are not equally affected by iron shortage. These arguments may be subjected to testing.

**Conclusion**
The occurrence of hereditary hemoglobin disorders in Bangladesh has been known for long time although the data is limited. The actual magnitude of these hereditary hemoglobin disorders have been masked by nutritional deficiency anemias. As because, iron deficiency anemia is the commonest type of nutritional anemia and coexisting iron deficiency anemia with beta thalassemia trait and hemoglobin-E trait is also common here, so it is wise to perform appropriate and proper investigations when hypochromic and microcytic blood picture is seen. It will also therefore be important to avoid prolongation of such treatment in suspected iron deficiency without monitoring of the response by repeated blood counts; where the response is poor, additional tests will be required, including Hb electrophoresis to find out the phenotypic pattern of hemoglobin.

**References**