Iron deficiency anaemia in sickle cell disorders in India


National Institute of Immunohaematology (ICMR), Mumbai, *Regional Medical Research Centre (ICMR),
Bhubaneshwar, **Govt. Medical College, Nagpur, ***Bulsar Raktadan Kendra, Valsad, *Nilgiri Adivasi Welfare Association, Nilgiri & **Indian Council of Medical Research, New Delhi, India

Received January 2, 2007

**Background & objectives:** Iron deficiency anaemia (IDA) is uncommon in individuals with sickle cell disease (SCD) because of availability of an adequate iron source potentially from increased red cell turnover and from blood transfusions. Also, iron deficiency anaemia can often go unnoticed because the sickle cell disease patients are already anaemic. Iron deficiency in sickle cell patients may result in lowering the intracellular haemoglobin concentration and this may ameliorate sickling. The present study was undertaken to determine the prevalence of iron deficiency anaemia and the response of iron supplementation in sickle cell disorders in tribal population of the four States viz. Maharashtra, Gujarat, Orissa and Tamil Nadu.

**Methods:** A total of 8434 individuals (7105 AA, 1267 AS and 62 SS) were tested for zinc protoporphyrin/haem (ZPP/H) ratio and haemoglobin levels. Twenty two sickle cell anaemia (SS), 47 sickle cell trait (AS) and 150 normal control (AA) individuals who were iron deficient, were given iron therapy for a period of 12 wk and the laboratory investigations were repeated at the 13th wk.

**Results:** Sixty seven per cent of subjects with sickle cell anaemia and 26 per cent with sickle cell trait had elevated ZPP/H ratios (>80 µmol/mol) as against 22.8 per cent of normal individuals. The elevated ZPP/H ratios is an indicator of microcytic anaemia of iron deficiency. Following iron therapy, an improvement in the Hb levels and ZPP/H ratios was observed in both sickle cell disorders and normal individual cases.

**Interpretation & conclusions:** This study suggests that iron deficiency anaemia is an important problem in Indian sickle cell anaemia patients and iron supplementation should be given only in proven cases of iron deficiency anaemia.

**Key words** India - iron deficiency anaemia - sickle cell anaemia - zinc protoporphyrin
thalassaemias and sickle cell anaemia since there is a premature destruction of erythrocytes, which contributes towards iron stores\textsuperscript{2,3}. It is believed that iron released by haemolysis is available for reutilization and that iron deficiency is uncommon in these conditions. In a study done about three decades ago it was found that the serum iron levels in young Jamaican children were significantly increased compared with age and sex matched AA controls\textsuperscript{4}. Iron stores may also be increased by transfusions\textsuperscript{5,6}. However, contrary to the previous belief a report from USA suggested that iron deficiency was common than expected in untransfused sickle cell anaemia cases\textsuperscript{7}. In tropical countries, it is expected that iron deficiency will be seen in sickle cell disease cases.

The present work forms a part of the study on nutritional anaemia and gene frequency distribution of haemoglobinopathies in different primitive tribes in four States of India, i.e., Maharashtra, Gujarat, Orissa and Tamil Nadu. These tribes stay in hilly areas and remote places where the health care facilities are very poor. The importance of iron deficiency specially in children cannot be ignored and so also in women of child bearing age. An earlier study from Orissa reported on iron deficiency in sickle cell anaemia cases\textsuperscript{8} while the earlier study was hospital-based, the present work adapts a community-based approach. We undertook this study to determine the incidence of iron deficiency anaemia in sickle cell homozygous, heterozygous and normal subjects in the tribal populations of Maharashtra, Gujarat, Tamil Nadu and Orissa where sickle cell gene frequency is also very high and to see the effect of iron supplementation in these cases.

\textbf{Material \& Methods}

A total of 8434 individuals (6168 adults and 2266 children) from 289 villages of Maharashtra, Gujarat, Tamil Nadu and Orissa were studied during 2000-2004. The villages were selected as the study unit in different tribal areas by PPS (population proportion to size). Of these 62 were homozygous (SS) for sickle cell anaemia (age 2-66 yr), 1267 were sickle cell heterozygotes (AS) (age 2-90 yr), while 7105 were normal without any haemoglobinopathies (age 2-96 yr). The male female ratio was 1:16, 1:13 and 1:12 in the SS, AS and AA, respectively. None of the sickle cell anaemia patients had been transfused earlier. Blood samples (2-3 ml) were collected in EDTA after taking informed consent. Haemoglobin phenotype was determined by automated HPLC using the Biorad Hb variant testing system (Biorad Laboratory, Hercules, California USA) using the $\beta$-thalassaemia short programme. RBC indices and zinc protoporphyin/haem (ZPP/H) ratio were measured on the Sysmex K-1000 cell counter (Japan) and ProtoFluor-Z Hematofluorimeter (Helena Laboratories, UK) respectively. The diagnosis of iron deficiency anaemia was established based on the following criteria: children (<18 yr)- Hb <11 g/dl and ZPP >80 $\mu$mol/mol; adult male (>18 yr)- Hb <13 g/dl and ZPP >80 $\mu$mol/mol; adult female (>18 yr)- Hb <12 g/dl and ZPP >80 $\mu$mol/mol.

Twenty two sickle cell anaemia (SS), 47 sickle cell trait (AS) and 150 normal (AA) controls who were found to be iron deficient were given 3 mg/kg/day elemental iron supplementation in the form of ferrous sulphate for a duration of 12 wk. This dose was decided with the suggestion of the experts while formulating the project. All the laboratory investigations were repeated at the 13\textsuperscript{th} wk. A questionnaire was used to assess the work efficiency after iron therapy.

The controls were the subjects without any haemoglobinopathies and were randomly selected from the population studied.

The study protocol was approved by the local ethics committee of participating institutions and also by the Central Ethics Committee of the ICMR.

\textbf{Statistical analysis}: Paired t test was used to compare the values before and after iron supplementation.

\textbf{Results}

The prevalence of IDA in sickle homozygotes (SS), sickle heterozygotes (AS) and normal individuals (AA) are shown in Table I. Iron deficiency anaemia was found to be very high in sickle cell homozygous

<table>
<thead>
<tr>
<th>Hb phenotypes</th>
<th>Adult</th>
<th>Children</th>
<th>Total IDA No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total tested</td>
<td>IDA</td>
<td>Total tested</td>
</tr>
<tr>
<td>SS</td>
<td>27</td>
<td>19</td>
<td>35</td>
</tr>
<tr>
<td>AS</td>
<td>925</td>
<td>237</td>
<td>342</td>
</tr>
<tr>
<td>AA</td>
<td>5216</td>
<td>1182</td>
<td>1889</td>
</tr>
<tr>
<td>Total</td>
<td>6168</td>
<td>1438</td>
<td>2266</td>
</tr>
</tbody>
</table>
cases (67.7%) as against sickle cell trait (26.2%) and normal controls (22.8%). Table II shows the initial haematological parameters of SS, AS and AA subjects who had IDA. No significant difference was observed in any of the parameters between these three groups. However, the mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) levels were slightly higher in SS patients whereas ZPP/H ratio was higher in AS cases. Two hundred nineteen subjects (22 SS, 47 AS and 150 AA) were given iron therapy. Of these 9 SS, 18 AS and 50 AA individuals did not take the prescribed medication and were excluded for the analysis. A significant improvement in haemoglobin levels and a decrease in ZPP/H ratio has been observed in both SS (P<0.05), AS (P<0.01 and 0.05) and AA (P<0.001) cases (Table III). An improvement in work efficiency was observed in all SS homozygous cases after intervention.

**Discussion**

Measurement of the ZPP/haem ratio is a useful field method for detection of iron deficiency in the population. However, presence of malarial parasites in the blood or unidentified fluorescent substance in plasma may elevate ZPP/haem ratio in a minor proportion. For accurate diagnosis of iron deficiency, tests like measurement of serum iron and/or serum ferritin and total iron binding capacity are required. Nevertheless, by using ZPP/haem ratio, iron deficiency anaemia has been reported in the population study as well as in common hereditary haemoglobin disorders. Earlier studies comparing ZPP and serum ferritin have shown that the sensitivity and specificity of ZPP varies from 60 to 98 per cent and 55 to 95 per cent respectively.

In an earlier study higher MCV and MCH levels among the Jamaican SS patients have been reported as compared to the normal (AA) controls. We have also found a similar observation in our study.

In the present study, iron deficiency anaemia was found to be more common in sickle cell anaemia patients as compared to sickle cell trait and normal controls and this could be due to low dietary intake during vasoocclusive crisis, infections and malabsorption. The reduced frequency of iron deficiency anaemia in sickle cell trait has been reported earlier and could be explained by increased iron absorption or alternatively by reduced iron requirement and a lower risk of discrepancy between iron supply and demand. There have been a few reports on the presence of iron deficiency in sickle cell disease. In 1981 Vichinsky et al reported that 16 per cent of non transfused sickle cell anaemia patients had iron deficiency. In Nigeria, iron deficiency anaemia has been reported in 31 per cent sickle cell anaemia children while in Jamaica 8.5 per cent of HbSS and HbSC children had iron deficiency anaemia. Earlier studies from Orissa, India showed a 23 per cent incidence of iron deficiency anaemia in sickle cell anaemia patients. However, this was a hospital-based study whereas the present study is a community-based study in remote tribal areas. The populations in these two studies were quite different including their food habits and dietary intakes. This could be the reason for the difference in prevalence rate.

**Table II. Initial haematological parameters in SS, AS and AA with iron deficiency anaemia**

<table>
<thead>
<tr>
<th>Hb phenotypes</th>
<th>Hb (g/dl)</th>
<th>MCV (fl)</th>
<th>MCH (pg)</th>
<th>MCHC (%)</th>
<th>ZPP/H (μmol/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS (n=42)</td>
<td>8.7 ± 2.5</td>
<td>72.5 ± 8.4</td>
<td>23.3 ± 2.6</td>
<td>32.2 ± 2.4</td>
<td>145.0 ± 60.5</td>
</tr>
<tr>
<td>AS (n=332)</td>
<td>9.9 ± 2.2</td>
<td>63.5 ± 8.9</td>
<td>19.8 ± 3.6</td>
<td>31.0 ± 3.0</td>
<td>152.7 ± 92.5</td>
</tr>
<tr>
<td>AA (n=1627)</td>
<td>9.9 ± 2.0</td>
<td>66.4 ± 8.5</td>
<td>20.3 ± 3.9</td>
<td>30.6 ± 6.1</td>
<td>150.6 ± 85.8</td>
</tr>
</tbody>
</table>

Values are mean ± SD

MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; ZPP/H, zinc protoporphyrin/haem ratio

**Table III. Hb and ZPP/H ratio in SS, AS and AA cases before and after iron supplementation**

<table>
<thead>
<tr>
<th>Hb phenotypes</th>
<th>Hb (g/dl)</th>
<th>ZPP/H (μmol/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Follow up</td>
</tr>
<tr>
<td></td>
<td>Initial</td>
<td>Follow up</td>
</tr>
<tr>
<td>SS (n=13)</td>
<td>7.3 ± 2.2</td>
<td>9.3 ± 2.49*</td>
</tr>
<tr>
<td>AS (n=29)</td>
<td>8.2 ± 1.99</td>
<td>9.6 ± 1.77**</td>
</tr>
<tr>
<td>AA (n=100)</td>
<td>8.4 ± 1.55</td>
<td>9.8 ± 1.56***</td>
</tr>
</tbody>
</table>

Values are mean ± SD

P*<0.05, **<0.01, ***<0.001 compared to initial values
Sickle cell patients with severe iron deficiency could be a suitable group for studies on iron therapy. In a clinical study, Peterson et al. demonstrated that patients who received iron supplementation had a significant rise in hemoglobin concentration. Powers et al. reported on children with severe iron deficiency and also demonstrated a response to iron therapy. Absence of bone marrow iron stores has been reported in children with sickle cell anemia; however, the response to iron supplementation has not been elucidated properly. In the present study, it has been observed that iron supplementation can significantly improve the Hb and ZPP/H ratio in both sickle cell disorders and normal individuals. The work efficiency and improvement in general conditions also occurred in these patients as evidenced by a questionnaire. Although the cases who did not take the iron supplementation regularly were excluded from the analysis, yet the total compliances in these primitive tribal groups could not be ensured.

It has been hypothesized that iron deficiency might be beneficial to the sickle cell patient by reducing the percentage of sickle cells, thus reducing painful crisis. We suggest that iron deficiency anemia is a potential problem in our sickle cell patients and iron supplementation should be given only in proven cases of iron deficiency anemia to improve their general condition and work efficiency.

Acknowledgment

Authors acknowledge the financial support received for this multicentric study from Ministry of Health & Family Welfare, Government of India, New Delhi, and thank the staff of different centres for their technical support.

References


Reprint requests: Dr Dipika Mohanty, National Institute of Immunohaematology (ICMR), 13th Floor, New Multistoried Building K.E.M. Hospital Campus, Parel, Mumbai 400 012, India
e-mail: mohantydipika@yahoo.com