Deficiency of folic acid, vitamins B 12 and their correlation with ferritin in childhood β-thalassemia major

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Abstract

To estimate the levels of folic acid, vitamin B12 and their relationship with ferritin in β-thalassemia major children. Thirty children with β-thalassemia major in the age group of 4 to 8 years were studied for estimation of serum ferritin, folic acid and vitamin B12 over a period of one year. These tests were determined by means of Immulite 1000 analyzer. The statistical analysis of β-thalassemia major group compared with normal control group, showed significant (p<0.001) decreases in serum folic acid, vitamin B12 levels whereas increased concentration of ferritin in all 30 study subject. Negative and significant correlations were observed between ferritin and folic acid, and vitamin B12 in β-thalassemia major group. The present study found a significant association between ferritin and folic acid, vitamin B12 deficiency in children with β-thalassemia.

Key words: Ferritin, Folic acid, Vitamin B12, β-thalassemia major

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Introduction

β – Thalassemia is the most common genetically transmitted hematological disorder in Indian children [1]. The thalassemia syndromes are a heterogeneous group of inherited disorders characterized by decreased either β or α globin chain synthesis. β homozygous thalassemia state presents with variable degree of anemia from early childhood and are generally transfusion dependent, a condition, clinically known as thalassemia major [2].

Vitamin B12 and folic acid are nutrients essential to hematopoiesis. Megaloblastic anemia is almost always due to lack of one of these two vitamins. Vitamin B12 deficiency can also results in severe neurological impairment. Ferritin is a 450 kDa protein consisting of 24 subunits that is present in every cell type [5]. Serum ferritin levels are measured in patients as part of the iron studies workup for anemia. The ferritin levels measured have a direct correlation with the total amount of iron stored in the body including cases of anemia of chronic disease [6]. Ferritin is also used as a marker for iron overload disorders, such as hemochromatosis and porphyria in which the ferritin level may be abnormally raised [7]. We studied the concentrations of serum ferritin, folic acid and vitamin B12 concentrations in patients with β - thalassemia.

Material and Methods

This study was carried out at Department of Biochemistry, Grant Medical College and Sir J.J. Group of Government Hospitals, Mumbai. All participants completed a medical history form and provided informed consent. 30 children with β-thalassemia major in the age group of 4 to 8 years were studied for estimation of serum ferritin, folic acid, and vitamin B12 over a period of one year. The control consists of 30 age-sex matched healthy subjects was someone who had never anemia.
Inclusion criteria were normal renal and liver function. Exclusion criteria were use of medications (therapy involving iron chelating, carbamazepine, phenytoin, 6-azauridine, anthopterin, antifolates, anticonvulsant agents, tamoxifen, and theophylline), diabetes mellitus, cancer, anemia, and systemic illness. The Institutional Ethical Committee at the Grant Medical College and Sir J.J. Group of Government Hospitals, Mumbai, India, approved the study.

**Blood sample collection**

Venous blood samples were collected in test tube with aseptic precautions. After 2 hours of collections sample was centrifuged at 3000 rpm for 5 minutes. Serum was separated and collected in polythene tube with cork. The sera with no sign of hemolysis used for the analysis of ferritin, folic acid and vitamin B12.

**Biochemical Analysis**

Serum ferritin concentration was measured by Solid phase, two site, and chemiluminescent immunometric assay. Serum folic acid estimated by the method of boil, competitive, liquid-phase, ligand-labeled, protein binding chemiluminescent assay with in situ immobilization, and with an anti- ligand detection system. The solid phase, a polystyrene bead enclosed within an Immulite test unit, is coated with murine monoclonal antibody specific for folic acid binding protein [9]. Serum vitamin B12 concentration was evaluated by solid phase, competitive chemiluminescent assay method [10]. We used fully automated enzyme amplified chemiluminescent immunoassay based Immulite 1000 analyzer. Measurement of these blood parameters by using commercial kits from Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA.

**Statistical Analysis**

Numerical variables were reported in terms of mean and standard deviation. Statistical analysis of results was done by normal ‘z’ test. In this analysis, variables showing p value less than 0.05 and 0.001 were considered to be statistically significant and highly significant respectively. Pearson correlation test was used to test.

**Results**

Table 1 depicts changes in serum profile when control group was compared with study group. As can be seen, significant decreases (p< 0.001) were observed in serum folic acid and vitamin B12 whereas, ferritin levels showed significant increase (p < 0.001).

**Table I. Serum folic acid, vitamin B12 and total homocysteine in controls and β-thalassemia patients.**

<table>
<thead>
<tr>
<th>Biochemical Parameters</th>
<th>Control (n=30)</th>
<th>β-thalassemia Patients (n=30)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferritin (ng/mL)</td>
<td>177 ± 24.2</td>
<td>3746 ± 1909</td>
<td>0.0001</td>
</tr>
<tr>
<td>Vitamin B12 (pg/mL)</td>
<td>409 ± 112</td>
<td>245 ±5 7.7</td>
<td>0.0001</td>
</tr>
<tr>
<td>Folic acid (ng/mL)</td>
<td>12.2 ± 2.10</td>
<td>9.02 ± 1.53</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

The values are presented in mean ± S.D. **P < 0.001.**
Table 2 showed that there were negative and significant correlations between serum ferritin and folic acid, and vitamin B₁₂ in study subjects. Correlation between serum ferritin and folic acid, and vitamin B₁₂ in β-thalassemia patients showed in Figure 1 and 2 respectively. Correlation coefficient® of them is highly significant (p < 0.001).

Table II. The correlation between serum ferritin and folic acid, and vitamin B₁₂ in β-thalassemia patients.

<table>
<thead>
<tr>
<th>Biochemical Parameters (n=30)</th>
<th>r</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferritin Vs Folic acid</td>
<td>-0.4836</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Ferritin Vs Vitamin B₁₂</td>
<td>-0.7245</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Figure 1. Correlation between serum ferritin and folic acid.
**References**


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