ABSTRACT

**Background:** β-Thalassemia major is considered to be one of the most common inherited hemolytic anemia. Enhanced years of survival of thalassemia have led to unmasking related complications related to alterations in certain trace elements like magnesium, calcium, phosphorus, copper, zinc etc.

**Objective:** Present study was conducted to evaluate the effect of iron chelation therapy and blood transfusion on certain trace elements (Magnesium, Calcium, Phosphorus, Copper, Zinc) in β-thalassemic patients on chelation therapy more than one year.

**Materials and Methods:** In the present cross sectional study, 100 β-thalassemic patients receiving chelation therapy for atleast 1 year were recruited from Civil Hospital Ahmedabad, Gujarat during February, 2017 to December, 2018 and equal number (n=100) of healthy subjects were taken as a control group in the age range of 8 to 15 years of both sexes (male & female). The levels of serum magnesium, calcium, phosphorus, zinc, and copper in serum were analyzed and results were correlated with normal healthy subjects.

*Corresponding author: E-mail: drkulidip08@gmail.com*
Results: A significant increase in serum copper (P≤ 0.01) and phosphorus (P≤ 0.001) were observed levels while a significant (P≤ 0.05) fall in magnesium, calcium and zinc levels recorded in β-thalassemic patients in comparison to healthy control subjects.

Conclusion: Aforementioned observations suggested that fluctuations in the trace elements levels in β- thalassemic children receiving blood transfusion and iron chelation therapy could leads to different complications like hemolyzed red cells, infections & hemochromatosis renal damage, hypoparathyroidism etc. if remains untreated. Hence routine assessment of these elements is recommended for better management.

Keywords: β- Thalassemia; magnesium; calcium; phosphorus; copper; zinc.

1. INTRODUCTION

Thalassemia, a genetic disorder of hemoglobin synthesis characterized by reduced or lack of production of one or more globin chains. The thalassemias are types of hereditary anemias occur due to mutations, which affect the synthesis of the globin chains, a protein component of hemoglobin. Thalassemias, the most common genetic diseases become a massive public health problems in many parts of the world [1] that encountered practically in every ethnicity and geographic location in the world, however, they are most common in the Mediterranean, the equatorial, and near equatorial regions of Africa and Asia [2,3,1,2].

The complications of thalassemia major in children are anemia, poor growth and abdominal enlargement due to hepatosplenomegaly. Bony complications secondary to medullary hyperplasia results in widening of diploic spaces, thinning of cortex leading to frontal bossing, prominence of upper incisors and separation of orbit. Over past 30 years, multiple transfusion therapy in these patients is associated with significant systemic disturbances because of iron overload in spite of different chelation modalities [3].

Biochemical alteration in trace elements as serum magnesium, calcium, phosphorus, zinc, and copper are a subject that should be investigated as it may be a precipitating factor for many complications as stunted growth, hair changes, delayed puberty and psychological changes. Zinc has an important role in growth and sexual development. Chronic zinc deficiency in thalassemia major may be multifactorial due to hyperzincuria, high ferritin levels, or secondary to chelation therapy [4,5].

Blood transfusions are necessary to prevent complications and also to maintain pre-transfusion hemoglobin levels between 9.5 g/dl to 10.5 g/dl. However, humans cannot excrete higher amount of iron, so regular blood transfusion cause iron overload. Extra iron get deposited in the liver, heart, pancreas, thyroid, parathyroid, adrenal, renal medulla, bone marrow, and spleen. This parenchymal iron deposition is the one of the cause of morbidity and mortality in the β-thalassemias. The management of severe forms of the β-thalassemia diseases includes: regular transfusion of blood, removal of overloaded iron from blood with chelating agents such as deferoxamine, deferiprone or deferasirox and splenectomy when rate of transfusion is increasing [6].

There are relatively a few studies on trace elements including magnesium, calcium, phosphorus, zinc and copper together in the thalassemic patients receiving long time iron chelation therapy and blood transfusion in India. So, the present study was designed to evaluate the effect of iron chelation therapy and blood transfusion on trace elements like magnesium, calcium, phosphorus, copper and zinc in children suffering with β- thalassemia.

2. MATERIALS AND METHODS

A cross sectional study was conducted on 100 known cases of β- thalassemia receiving blood transfusion & chelation therapy of iron for more than one year in the age range of 8 to 15 years of both sexes (Male & Female) during the period from February 2017 to December 2018. The β-thalassemia patients admitted in Paediatric ward of Civil Hospital, Ahmedabad were taken for the study and equal number of normal healthy subjects of both sexes in the same age range of 8-15 were selected from paediatric OPD (children who visited OPD for their vaccination as well as children who are visiting/accompanied with the other patient and fulfilled the inclusion as well as exclusion criteria for controls) at Civil Hospital, Ahmedabad.
Inclusion Criteria for cases: 100 confirmed cases of β-Thalassemia major of both sex in the age range of 8 to 15 years old receiving chelation therapy for at least 1 year.

Inclusion Criteria for control: 100 healthy children of both sexes in the age range of 8 to 15 years old. The healthy children not suffered from any chronic illness and also not received blood transfusion in recent past.

Exclusion criteria for both cases and controls: We excluded children aged less than 8 years and more than 15 years. The children with liver disease, cardiac disease, renal disease, protein energy malnutrition, and trauma like surgical, burns, fractures, malignancy: lymphoma, carcinoma, sarcoma, leukemia were excluded.

Collection and processing of blood samples: 5ml of venous blood was taken from all subjects after 12 hour overnight fast in a dry disposable syringe under all aseptic conditions by venepuncture in the antecubital vein in a sterile, dry acid washed vial for biochemical assays.

Preparation of serum: The blood was allowed to stand for half an hour. After clot formation, the supernatant was centrifuged for 15 minutes at 3000 rpm. All the samples were processed for trace elements analysis.

Biochemical Assays: The transelement like magnesium, calcium, phosphorus, zinc and copper in serum of both groups were estimated by using the commercially available kits manufactured by Transasia Pvt. Ltd by colorimetric method in ERBA XL-640 Fully Auto Analyzer at Hi-Tech Biochemistry Laboratory, Civil Hospital, Ahmedabad.

Statistical Analysis: The Master chart was prepared using Excel 2010 software. The data was expressed as Mean ± SD and analyzed with the SPSS 16.0.7 statistical software package. Differences between the β-thalassemic children and healthy controls subjects were evaluated using the Student's independent samples “t” test. Differences were considered statistically significant at p <0.05.

3. RESULTS

Anthropometric Profile Status: 100 patients of β-thalassemic and 100 healthy children with matched age and sex as a control group were included in study. On analysis of all patients with thalassemia major, we found the mean ages in patients with thalassemia were comparable to those in group control. The mean ages of patients with thalassemia and control were 9.86 ± 6.21 and 8.9 ± 5.68 years, respectively (P<0.005). Sexes were comparable between the two study groups. Male/female ratio was 28/22 in patients with β-thalassemia compared with 24/26 in healthy controls.

Trace Elements: Serum magnesium, calcium, phosphorus, zinc and copper concentrations in both thalassemia major patients and control summarized in Table 2. A significant increase in serum copper by 55.58% (P≤ 0.01) from 93.84 ± 9.2 µg/dL to 146.00 ± 6.90 µg/dL and phosphorus by 104.44% (P≤ 0.001) from 4.5 ± 2.62 mg/dL to 9.2 mg/dL ± 2.02 were observed levels while a significant fall in magnesium by 57.89% (P≤ 0.01) from 1.9 ± 0.9 mg/dL to 0.80 ± 0.05 mg/dL, calcium by 51.51% (P≤ 0.05) from 9.92 ± 1.4 mg/dL to 4.81 ± 1.90 mg/dL and zinc 41.81% (P≤ 0.05) from 93.21 ± 8.9 µg/dL to 54.23 ± 8.62 µg/dL levels were recorded in β-thalassemic patients with respect to healthy control subjects.

4. DISCUSSION

A present case control study was conducted to correlate the levels of trace elements like magnesium, calcium, phosphorus, copper, zinc among the children suffered with thalassemia major and healthy controls taken from civil hospital Ahmedabad of Gujarat state, India as alterations in the trace elements play a significant role in the pathophysiology of various diseases. A significant (P≤ 0.01) increase in serum Cu levels in β-thalassemic patients receiving the chelating therapy was recorded in the present study. Hypercupremia in β-thalassemic patients after long chelating therapy could lead to hemochromatosis this could be due to antagonistic effect of zinc deficiency as observed in the present study (Table 2). A significant fall in zinc levels in β-thalassemic patients observed w.r.t. control subjects in the present study could be greatly increased copper absorption via the gastrointestinal tract. This negative correlation can be attributed to that elements having similar orbital valency might compete for specific binding sites on proteins involved in their absorption and perhaps also during de novo synthesis of metal isoenzyme [7] and/or a significant fall in serum Zn levels in β-thalassemic patients could be due to either an excessive release from hemolized red cells, desferrioxamine therapy, or undernutrition [8,9].
**Table 1. Changes on Anthropometric profile in β-thalassemic children and healthy controls subjects**

<table>
<thead>
<tr>
<th>Anthropometric Profile</th>
<th>Normal Healthy control subjects (Mean ± S.D.)</th>
<th>β-Thalassemic Children (Mean ± S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male (n= 54) Female (n= 46)</td>
<td>Male (n= 58) Female (n= 42)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>9.1 ± 5.12a 8.7 ± 6.24</td>
<td>9.15 ± 5.94 9.57 ± 6.48</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>72.16 ± 6.87 69.12 ± 7.25</td>
<td>70.17 ± 4.14 68.14 ± 1.25</td>
</tr>
<tr>
<td>Body Weight (Kg.)</td>
<td>12.24 ± 3.54 10.91 ± 5.21</td>
<td>10.12 ± 5.24 8.21 ± 2.41</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>23.54 ± 5.25 21.12 ± 6.21</td>
<td>18.16 ±3.95 17.61 ± 4.54</td>
</tr>
<tr>
<td>Fasting Blood Glucose (mg/dL)</td>
<td>78 ± 9.81 77.68 ± 8.58</td>
<td>82.98 ± 6.44 79.18 ± 7.53</td>
</tr>
<tr>
<td>Systolic Blood Pressure (mmHg)</td>
<td>109.00 ± 7.00 102 ± 6.00</td>
<td>101.00 ± 4.00 108.00 ± 3.00</td>
</tr>
<tr>
<td>Diastolic Blood Pressure (mmHg)</td>
<td>79.00 ± 6.00 81.00 ± 8.00</td>
<td>78.00 ± 8.00 79.00 ± 6.00</td>
</tr>
</tbody>
</table>

*Values are expressed as Mean ± S.D.*

**Table 2. Changes in certain trace elements (magnesium, calcium, phosphorus, zinc & copper) in β-thalassemic children and healthy controls subjects**

<table>
<thead>
<tr>
<th>Biochemical Assays</th>
<th>Healthy Controls Subjects (n=100)</th>
<th>β-Thalassemic Children (n=100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magnesium (mg/dL)</td>
<td>1.9 ± 0.10</td>
<td>0.80 ± 0.050(-57.89)</td>
</tr>
<tr>
<td>Calcium (mg/dL)</td>
<td>9.92 ± 1.40</td>
<td>4.81 ± 1.90(-51.51)</td>
</tr>
<tr>
<td>Phosphorus (mg/dL)</td>
<td>4.5 ± 2.62</td>
<td>9.2 ± 2.02(+104.44)</td>
</tr>
<tr>
<td>Zinc (µg/dL)</td>
<td>93.21 ± 8.90</td>
<td>54.23 ± 8.62(-41.81)</td>
</tr>
<tr>
<td>Copper (µg/dL)</td>
<td>93.84 ± 9.20</td>
<td>146.00± 6.90(+55.58)</td>
</tr>
</tbody>
</table>

*Values are expressed as Mean ± S.D.*  
*Values in parentheses represent percentage changes w. r. t. normal healthy subjects.*  
*P≤ 0.05   **P≤ 0.01   ***P≤ 0.001*

A significantly fall in serum Calcium, Mg levels while a significant increase in phosphorus levels observed in the thalassemic patients with respect to healthy control subjects (Table 2) are in agreement literature reports that hypocalcaemia in thalassemic patients could be multifactorial either due to hypoparathyrodisism due to iron overload and/or iron deposition in the parathyroid or secondary to the chelation therapy/or for delay in starting iron chelating therapy, poor absorption from intestine and poor compliance with the therapy in some of our patients [10,11] Furthermore, a significant fall in serum Mg levels in patients with β-thalassemic patients could be due to lower thyroid hormones resulted from iron overload [12].

**5. CONCLUSION**

A formentioned observations suggested that fluctuations in the trace elements levels in β-thalassemic children receiving blood transfusion and iron chelation therapy could leads to different complications like hemolyzed red cells, infections and hemochromatosis renal damage, hypoparathyroidism and hypoparathyroidism etc if remains untreated. Hence routine assessment of these elements is recommended for better management. In addition, further large-scale studies are recommended.

**CONSENT AND ETHICAL APPROVAL**

The study protocol as approved by the Institutional Ethics Committee. The study details & potential risks and benefits were explained to individuals taking part in the study and at least. A written one attendant informed consent was obtained from subjects before entering into the study in the vernacular language. Moreover, confidentially of the data will be maintained.

**ACKNOWLEDGEMENTS**

Authors are thankful to all the participants for giving us opportunity for being a part of this study.

**COMPETING INTERESTS**

Authors have declared that no competing interests exist.
REFERENCES


© 2019 Parmar et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here: http://www.sdiarticle4.com/review-history/53338