Growth Status and Serum Zinc Level in Patients with Haemoglobin –E-ß Thalassemia
AKM AMIRUL MORSHED1, SHAHNOOR ISLAM2, AFQUL ISLAM3

Abstract
Background: Hb- E- ß thalassemia is a major congenital hematological disease of Bangladesh. These patients have poor growth and delayed maturation mainly due to iron overload but zinc deficiency also has been suggested as a contributing factor.

Objectives: To investigate and compare the serum zinc of Hb E ß thalassemia patients and normal children and to see the relationship between serum zinc and growth status.

Methods: This cross sectional study on Hb –E ß thalassemia was undertaken in the out patient department of Paediatric Haematology and Oncology department of Bangabandhu Sheikh Mujib Medical University from July 2009- May 2010. Total 31 children with Hb- E- ß thalassemia were enrolled for this study and matched 30 healthy children were taken as control. History of the cases were taken thoroughly and examination were done by the investigator himself. Anthropometry were measured properly and plotted in Centre for Disease Control (CDC) chart. Three ml (3 ml) of venous blood samples were drawn from both patient and control. Later on the blood samples were centrifuged and serum was stored at – 20c till analysis for serum zinc level. Serum level of zinc was measured by using Flame Atomic Absorption Spectrophotometry following standard procedure.

Results: Mean serum zinc level in thalassemic group and control group were 97.4µg/dl (SD-18.4) and 99.6µg/dl (SD-18.7) respectively. There was no significant difference between two groups (p=.47). Mean percent of 50th centile of weight achieved was 75.9% in cases and 81% in control. Similarly mean percent of 50th centile of height achieved was 89% in cases and 93% in control. There was significant difference in height for age between both the groups (p=.05).

Conclusion: Serum zinc level did not significantly changed in thalassemic group and control group children. But there were significant stunting in Hb –E ß thalassemia patients and no significant difference was found between these children in terms of weight for age.

Key Words: Hb –E ß thalassemia, zinc, growth.

Introduction
Thalassemia is the commonest hereditary hematological disorder1. Thalassemias are prevalent in populations that evolved in humid climates where malaria was endemic. It affects all races, as thalassemias protected these people from malaria due to the blood cells easy degradation. Thalassemias are particularly associated with people of Mediterranean origin, Arabs, and Asians1. The Maldives has the highest incidence of thalassemia in the world with a carrier rate of 18% of the population. The estimated prevalence is 16% in Cyprus, 1% in Thailand, and 3-8% in populations from Bangladesh, China, India, Malaysia and Pakistan2. There are also prevalences in descendants of people from Latin
America and Mediterranean countries (e.g. Greece, Italy, Portugal, Spain, and others). A very low prevalence has been reported from people in Northern Europe (0.1%) and Africa (0.9)\textsuperscript{2}.

The World Health Organization (WHO) estimates that in Thailand at least 100,000 new cases of Hb E β-thalassemia are expected in the next few decades. Among all thalassemia syndrome Hb-E-α thalassemia is the most common in our country and in south east Asia\textsuperscript{3,4}. Hemoglobin E Syndrome carrier frequency in Bangladesh is 6\%\textsuperscript{5,6}.

Zinc is an essential trace element that is required for normal intestinal mucosal integrity, skeletal growth, sodium and water transport and immune function. Clinical zinc deficiency is prevalent in Bangladesh, particularly among malnourished children and who are suffering from chronic diseases. Zinc is an essential trace metal for the normal function of many enzymes involved in cell division and DNA protein synthesis in mankind. Zinc deficiency is associated with several clinical manifestations, such as growth retardation, delayed wound healing skin changes, hypogonadism, glucose intolerance, anemia and reduced leucocyte function. Patients with thalassemia major not receiving regular chelation treatment or blood transfusion also have serum zinc values below normal and they have increased urinary zinc excretion\textsuperscript{7}. Some study in India had shown that zinc deficiency aggravate anemia\textsuperscript{8}.

It has been recognized for several decades that children with Hb E- α-thalassemia have poor growth and delayed puberty\textsuperscript{9,10,11}. Iron overload is the main reason for these disorders, but zinc deficiency has been suggested as a contributing factor in these patients. Trace minerals, especially zinc, are very important for human growth, puberty and immune system\textsuperscript{12}. On the other hand, iron chelation therapy with desferrioxamine, which is necessary for major thalassemic patients, increases urinary excretion of zinc\textsuperscript{13}. Zinc status in patients with thalassemia has been under debate\textsuperscript{14}. The studies of Shamshirsaz, Bekheilia and Arcasoy showed that serum zinc level is less than normal in 80-100% of the patients\textsuperscript{15,16,17}. However, in other studies serum zinc level was not statistically different in thalassemic subjects compared with a control group and even Donmà found higher values for hair zinc in patients with thalassemia\textsuperscript{18}. As there have been some reports indicating positive effects of oral zinc supplementation on height velocity in children with definite zinc deficiency\textsuperscript{12}, some thalassemic patients in our country receive zinc supplement, even though its necessity and efficacy are vague. Several studies have shown the status of zinc in Beta thalassemia major but no study specifically done on Hb–E- α thalassemic patients. Considering this and different zinc status in different studies in thalassemic patients, the study was taken to investigate and compare serum zinc level in Hb-E- α thalassemic patients and normal individuals.

**Methodology**

Cross sectional study has been conducted at department of pediatric Hematology and Oncology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka and Thalassemia centre, Dhaka Shishu hospital, Sher-e-Bangla nagar, Dhaka from July 2009 to May 2010.

Inclusion criteria were diagnosed cases of Hb E- α Thalassemia patients of 2-12 years of age attending Outpatient department (OPD) of BSMMU and Thalassemia centre of Dhaka Shishu Hospital. Patients who have any associated medical conditions (e.g. Protein Energy Malnutrition (PEM), congenital cardiac disease, bronchial asthma, neurological disorders and haemoglobinopathies etc), children taking any dietary supplement containing zinc during the last month and acute febrile infected children based on physical examination at the time of blood sampling were excluded because zinc level reduce in febrile illness\textsuperscript{19}.

In this study, 31 patients with Hb E- α-thalassemia who were on regular blood transfusion in 2 (two) thalassemia centres in Dhaka were entered into this study from July 2009 to May 2010. Diagnosis of Hb- E- β thalassemia was based on peripheral blood smear evaluation and hemoglobin electrophoresis of the patients. Patients was excluded from this study if they had any underlying medical condition other than thalassemia, or any dietary supplement containing zinc during the last month because zinc level may increase if anyone intake it in this period of time. Patients who are febrile (oral temperature >38°C) or was acute infection based on physical examination at the time of blood sampling also excluded because zinc level reduce in febrile illness\textsuperscript{19}. Consent was obtained from their parents. Anthropometric data, time of diagnosis, time interval between blood transfusions, history of splenectomy, and patient medications collected by researcher himself. Patient’s height measured with a stadiometer and weight measured
with bathroom scale. Percentiles of height and weight were determined by growth chart provided by the CDC. Blood samples (3mL) were drawn from patients and then centrifuged and stored at -20°C until analyzed. Serum level of zinc was measured using flame atomic absorption spectrometry (Atomic absorption spectrophotometer Perkins Elmer model no 3110, Germany) in Analytical Chemistry laboratory of Atomic Energy Centre Ramna Dhaka. Serum samples were diluted (1:1) with de-ionized water. The viscosity of the diluted serum samples was then matched effectively with the working standard. The working standard solution was prepared by diluting a stock solution containing 1000 mg/ L of single element AAS grade standard with ultra pure water and 5% glycerin. A Varian (Varian, DuoAA240FS and AA280Z) atomic absorption spectrometer (AAS) equipped with fully integrated atomizers (viz. a burner system for flame atomization), was used for doing analysis.

Serum zinc level less than 60g/dL was regarded as low. Thirty age- and sex-matched healthy children from out patient department of BSMMU who have the same socioeconomic status were selected and their serum zinc level was analyzed.

Data was taken in well validated Questionnaire (prepared and permitted by supervisors and ethical committee of BSMMU) which was pre-tested by few samples in OPD of Paediatric Haematology and Oncology department of BSMMU. Data was processed by computer software SPSS Version 16 and results was analyzed by student’s t test, c² test and Anova test. Due to the economical constraints and time limit large sample could not taken and other investigations like feritin level and relationship between serum feritin and zinc could not established. This protocol was approved by the ethical committee of BSMMU. Informed consent had been taken from the parents.

Results
This study was done on special group of children whom are suffering from HbE- β Thalassemia attending in out patient department of Paediatric Haematology and Oncology, BSMMU and Thalassemia Centre of Dhaka Shishu Hospital. The study included 31 children suffering from Hb E-β thalassemia and 30 children are healthy age and sex matched controls who are not suffering from thalassemia. Mean age of this study was 79.8 months with SD 38.3 months range from 24-144 months in patients with HbE – β thalassemia and those in control group mean age was 74.4 months with SD-36.3, maximum 144 and minimum 24 months. Total 26(42%) children were male while 35(58%) children were female in this study. In Hb E- β thalassemia group 13(42%) cases were male while 18(58%) cases were female but in control group it was 13(43%) and 17(57%) respectively. Four children (7%) had come from a family with consanguinity of marriage while 57 children (93%) had come from family of non-consanguinous family. In 3(10%) children of Hb E-β thalassemia group were from family of consanguinity while it were only in 1(3%) in the control group. Twenty two (71%) cases of Hb E – β thalassemia had palpable liver and in 09(29%) cases liver was not palpable. Mean length of palpable liver was 2.9 cm ranging from 1cm to 8 cm. In case of spleen, there were 29(93%) cases came with palpable spleen with mean length of 6.8 cm ranging from 1 cm to 15 cm. Splenectomy was done in one case of Hb E – β thalassemia patient.

Mean weight of the children with Hb –E- β thalassemia was 17.4kg (SD-5.5, Mini-10; Maxi-30) while in control group it was 17.6kg(SD-5.9, Mini-10: Maxi-29). There was no significant difference of wt between two groups. According to the Gomez classification of nutrition mean weight for age in Hb –E- β thalassemia group was 75%. but on the other hand mean wt for age in control group was 81%. So there was no significant difference of nutritional status between two groups (p=.47).

<table>
<thead>
<tr>
<th>Group</th>
<th>Weight</th>
<th>Percent of 50th centile</th>
<th>p</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Mean(kg)</td>
<td>SD</td>
<td>Min (kg)</td>
</tr>
<tr>
<td>Hb-E-β</td>
<td>17.4</td>
<td>5.5</td>
<td>10</td>
</tr>
<tr>
<td>Control group</td>
<td>17.6</td>
<td>5.9</td>
<td>10</td>
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</tbody>
</table>
Mean height of the children with Hb –E- β thalassemia was 106 cm (SD-14.7, Mini-77; Maxi-141) while in control group it was 106.8 cm (SD-17.9, Mini-78: Maxi-140). There was significant difference of height for age between two groups. Mean height for age in Hb –E- â thalassemia group was 89% but on the other hand mean height for age in control group was 93%. So there was significant difference of height for age between two groups (p=0.05).

Among 31 thalassemia patients no patient was severely wasted and 1 patient was moderately wasted and rest patients were mildly wasted to normal. In control group 1 child was severely, 4 children were moderately wasted and rests were mild to normal.

Mean serum zinc level in Hb –E- β thalassemia group was 97.4 µg/dl and in control group it was 99.6 µg/. There was no significant difference of serum zinc level in Hb –E- β thalassemia group and control group (p=0.47).

In this study total sixty one children were included. Among them thirty one children were patients and thirty were control. Age and sex matched population who had no thalassemia or no major illness was taken as control population. The participating children were predominantly urban residents (around 60%)

In thalassemia patients the male: female ratio was roughly 2:3, which was not consistent with the study conducted in Bangladesh by Rahman & Jamal, where the same ratio was roughly 1:1. In our study mean age in the study group was approximately 80 months with a standard deviation of 38 months. The youngest and the oldest children were of 24 and 144 months respectively. These findings are almost consistent with the findings of Rahman & Jamal. However, Mehdizadeh and associates in a similar study reported a higher mean age in their study (164 ± 69.6 months).

The difference between Hb-E- β thalassemia and control groups in terms of consanguinity of marriage was negligible (10% vs. 3%) and is staggeringly low compared with Shiraj's study conducted in Iranian population where > 40% of patients of beta thalassemia major had consanguinity of marriage. In the absence of local data about consanguinity of marriage we cannot make any conclusion about it.

In the present study 93% and 71% of the patients with Hb-E- β-thalassemia exhibited palpable liver and spleen. One patient was found with previous splenectomy. But in a local study nearly all patients had enlarged liver and spleen and 3 patients presented with previous splenectomy. Our result differed from this study in both enlargements of liver and spleen which might be due to frequent blood transfusion in our cases.

Table-II

<table>
<thead>
<tr>
<th>Group</th>
<th>Height percent of 50th centile</th>
<th>p</th>
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<tbody>
<tr>
<td></td>
<td>Mean(cm) SD Mini(cm) Max(cm)</td>
<td>Mean(%) SD Mini Max</td>
</tr>
<tr>
<td>Hb–E–β Thalassemia gr.</td>
<td>106  14.7  77  141</td>
<td>89%   4.8  79% 97%</td>
</tr>
<tr>
<td>Control group</td>
<td>106.8 17.9 78 140</td>
<td>93%   2.5  89% 97%</td>
</tr>
</tbody>
</table>

There is no relation with serum zinc level and gender of the children. Mean zinc level in male was 99.6 (SD-19.7) and female it was 96.3 (SD-17.0). There are no relation in serum zinc level and different age group of the children (p=0.69). There is no relation between serum zinc level and different stratum of Gomez classification (p=0.2).

Discussion

In this study total sixty one children were included. Among them thirty one children were patients and thirty were control. Age and sex matched population who had no thalassemia or no major illness was taken as control population. The participating children were predominantly urban residents (around 60%)

In thalassemia patients the male: female ratio was roughly 2:3, which was not consistent with the study conducted in Bangladesh by Rahman & Jamal, where the same ratio was roughly 1:1. In our study mean age in the study group was approximately 80 months with a standard deviation of 38 months. The youngest and the oldest children were of 24 and 144 months respectively. These findings are almost consistent with the findings of Rahman & Jamal. However, Mehdizadeh and associates in a similar study reported a higher mean age in their study (164 ± 69.6 months).

Table-III

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum zinc level (µg/dl)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean SD Min Max</td>
<td></td>
</tr>
<tr>
<td>Hb–E–β Thalassemia</td>
<td>97.6 18.4 69 146</td>
<td>0.47</td>
</tr>
<tr>
<td>Control</td>
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Table-II

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<th>Group</th>
<th>Nutritional status of the cases (Height and Height for age)</th>
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<tbody>
<tr>
<td></td>
<td>Group Height percent of 50th centile</td>
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Mean height for age in Hb–E-β thalassemia group in our study was 89 ± 4% and that in control group was 93±4.8% with significant intergroup difference (p = 0.05). In Iranian and Thai studies mean heights for age were 96.2 ± 4.7% and 96 ± 4% respectively\(^\text{12,14}\). Apparently it seems that Iranian and Thai children with Hb–E-β thalassemia are in a better position than their counterpart in Bangladesh. But when the mean height for age of Iranian children was compared with their normal children the result was fairly comparable (mean height for age was 101.2 ± 23.6%) \(^\text{12}\).

The findings of the present study showed that the mean serum zinc level of Hb-E β thalassemia group was almost identical with that of the control group 97.4 ± 18.4 µg/dl vs. 99.6 ± 18.7 µg/dl (p = 0.47). Our study did not coincide with previous Iranian study where mean serum zinc level in beta thalassemia major children was significantly higher (108.16 ± 17.2 µg/dl) than that in normal children (93.56 ± 13.6 µg/dl) \(^\text{12}\). Sharply contrasting with these findings in Egyptian study showed that there was significantly lower level of zinc in thalassemic population than that in the normal children where serum zinc level was 88.3 ± 17.7 µg/dl vs. 113.5 ± 15.4 µg/dl respectively\(^\text{21}\). Al-Refaie et al reported that zinc level in thalassemic children was 88.4 ± 11.5 which was much lower than Iranian study\(^\text{7,12}\).

Although different investigators showed different results (some with lower and some with higher serum zinc level in Hb-E β thalassemic children compared to the control group) our study did not show any significant difference between Hb-E β thalassemic and control group children in terms of serum level of zinc.

**Conclusions**

From the findings of the study it can be concluded that there were significant stunting(height for age) in patients with Hb –E α thalassemia and no significant difference between Hb –E α thalassemia children and normal control children in terms of weight for age and serum zinc level.

**Recommendations**

Mean serum zinc level is within normal range in children with Hb–E β thalassemia and normal control children. So supplementation of zinc in children with Hb–E α thalassemia is not essential.

**References:**


