

Status of Iron, Magnesium and Zinc in β -Thalassemia Patients of Bangladesh

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Abstract

In this study, we attempt to evaluate the status of iron, magnesium and zinc, in patients with β -thalassemia major during regular blood transfusion and medication for iron chelation in Bangladesh. This study is so far the first time investigation to report on micronutrients status of β -thalassemia patients in Bangladesh.

One hundred and ten (110) β -thalassemic patients (mean age 4-16 yrs) with blood transfusion were randomly selected for blood serum collection. In addition 48 (mean age 4-25 yrs.) healthy control samples were collected randomly from different areas of Bangladesh. Iron, Magnesium, and, Zinc were analyzed by atomic absorption spectrophotometer (AAAnalyst 800). Results were statistically analyzed using SPSS v2.0 software.

Interestingly, mean serum iron level was significantly higher in β -thalassemia patients compared to control. The results showed that the iron ($p < 0.05$) increased in beta-thalassemia patients (3.76 ± 1.65 mg/L,) whereas this concentration was found to be normal (2.01 ± 1.13 mg/L) in control. Moreover the, the level of magnesium observed was higher than the normal value (2.28 ± 0.74 mg/L) compared to control (1.11 ± 0.57 mg/L) but zinc remains unchanged (2.41 ± 0.34 mg/L) as compared to control (2.24 ± 1.02 mg/L).

KEYWORDS: Atomic Absorption Spectrophotometer, Iron, Magnesium and Zinc, β -thalassemia

Introduction

Thalassemia is one of the most common monogenic hereditary disorders in human (Gibson et al., 2005). The disease is most common in Mediterranean, Middle East, North Africa and Asia. Almost 0.21% population suffers from this disorder in Bangladesh (IbnAyub et al., 2010; Olivieri et al., 1999). The most common type of disorder found in Bangladesh is β -thalassemia minor, and second is the HbE β -thalassemia (Gazi et al., 2011; Khan et al., 1999) and β -thalassemia major, which requires regular blood transfusions and desferrioxamine injection. Patients suffer from several disorders including metabolic, skeletal and retarded growth is common due to high concentration of iron storage in the body organs (Widad et al., 2003).

On the other hand magnesium and zinc are considered as important micronutrient which play vital role as cofactor for more than 200 enzymes (Donma et al., 1990).

Many studies report that patients with β -thalassemia have significantly higher level of zinc and iron and found no relationship with growth during blood transfusion (Wanpir et al., 2000). Other studies reported that thalassemia patients are at high risk of zinc deficiency due to various causes including hyperzincuria; high ferritin level and hepatic iron overload. Decreased level of zinc in both serum and plasma clinically resembles homozygous beta-thalassemia (Fuchset al., 1996).

Biochemical trace elements such as iron, magnesium, and zinc, have been analyzed in various populations for evaluating the correlation of growth on β -thalassemia patients (Das et al., 2004). Only few studies have been done in adult homozygous β -thalassemia patients to understand the status of micronutrients during blood transfusion. The disease is associated with severe anemia, jaundice, splenomegaly, extended bone marrow space and cardiomegaly. All these symptoms appear only after 3-6 months of age. Clinical symptoms are caused by hemolysis in the peripheral circulation and deposition of excess iron in tissues Iron overload is also occurs due to transfusional hemosiderosis and excess iron absorption in the gastrointestinal (Wolfgang et al., 2006).

The leading cause of death is due to iron overload in cardiac tissues (Papanikolaou et al., 2005). In the absence of chelating therapy, the accumulation of iron results in progressive dysfunction of heart, liver and endocrine glands. Another study states that iron chelation therapy with desferrioxamine proved to elevate the levels of copper and zinc in serum of Jordanian patients with α -thalassemia major (Suthipark et al., 1999).

In Bangladesh approximately 11.2 million thalassemia carrier has been identified with wide geographic variations. Out of which about 22.67% is β -thalassemia minor (Gazi et al., 2011). In this study we attempt to evaluate the status of major micronutrients (Fe, Mg and Zn) in β -thalassemia patients visiting the hospital.

Experimental Subjects

This study was carried out in collaboration with Thalassemia Foundation Hospital in Dhaka 2012-2013. The study population included 110 patients with β -thalassemia minor, intermediate. Also some β -thalassemia major patients were included who undergo periodical blood transfusion and treated with chelating agents. The tested group aged from 4-16 years. The diagnosis of β -thalassemia major were made based on the clinical, hematological and hemoglobin electrophoresis profiles and sequencing of HBB gene in some cases. All patients filled out standard questionnaires during the first visit of study and their medical histories were collected from the hospital records. Except minor patients all were dependent on blood transfusion 1-2 times per month. We also included 48 control healthy subjects (age group 4-25years) for this study. This study was approved by the ethical review board of University of Dhaka and Thalassemia Foundation Hospital, Dhaka.

Blood Collection

Approximately 3-5 mL of venous blood was collected just before the transfusion in the hospital under aseptic condition. Control blood was collected from different areas of Dhaka city. Blood was divided into two parts; 3mL blood was centrifuges at 2500 rpm and serum was collected for minerals analysis. The serum samples were stored at 4° C before analysis. The analysis of iron, magnesium and zinc were performed with AAS Flame.

Instrumental

The instrument AAnalyst 800 system was used for analysis of iron, magnesium and zinc by flame method. We used element specific lamp for each element wavelength. The high-light-throughput optical system of the AAnalyst 800, combined with a solid-state detector, provides the highest quality efficiency and signal-to-noise performance of any AAS system, with the acclaimed WinLab32™ control software, and real-time double beam optical system (Gazi et al., 2008; Kajanachumpol et al., 1997)

Material

We avoided the use of glassware for sample preparation. We used 50mL stoppered polypropylene volumetric flasks for flame system for the analysis. Automatic pipettes ranging from 50-100 uL with disposable pipette tips was used.

Sample Preparation: Serum samples were diluted 20-fold with deionized water.

Standard preparation: The standards used for zinc calibration curve were 0.5, 1.0, 2.0, 3.0 mg/L. For magnesium, and iron the standards used were 0.5, 1.0, 2.0, 3.0 mg/L respectively.

Statistics

All data were analyzed with SPSS v2.0 software. All the results were expressed as mean \pm SD. Student 't' test was applied for the comparison of data, $p > 0.05$ was considered as non-significant and $p < 0.05$ was considered as significant.

Results and Discussion

Thalassemia patient's history was collected from the hospital record. The blood group and hematological data of the experimental patients and the control groups are shown in Table 1 and 2. A significant observation was made from blood group. Most of our HbE β -thalassemia patients belong to B+ blood group (69.07%) next is O+ blood group (24.56%). A decrease of hemoglobin concentration in patients is observed when compared with control group. The analytical results of iron, magnesium, and zinc are shown in Table 3. The mean, standard deviation and t-test for iron, magnesium, and zinc have compared to control group.

In this study, samples were mixed type thalassemia. In Bangladesh, most prevalent type is HbE β -thalassemia and the next type is β -thalassemia minor. Less than <3% suffer from β -thalassemia major. This study revealed that beta thalassemia is more prevalent in

B+ and O+ blood group compared to control blood groups. This may be because most of the patients in our study were either B+ or O+ and only few samples are AB+. Further confirmation of blood group relationship to hematological disorder is required.

An increase in iron indices observed in patients may be due to hyper-hemolysis of RBC and also due to low hematocrit level and high ferritin content. Similar results were also observed in Mediterranean patients (Kuldeep et al., 2011). The higher serum iron concentration may be due to ineffective erythropoiesis and blood transfusion. The higher iron concentration on the other hand may affect the zinc and magnesium level in the blood and can lower the zinc concentration in thalassemia patients (Bashir et al., 2008). In contrast, this study showed normal zinc level in all thalassemia patients. The study indicates zinc deficiency in thalassemia patients who are on regular blood transfusion is rare and seems that routine zinc supplement is not required (Mahshid et al., 2008).

Similarly, the magnesium is considered an important micronutrient with highest amount in liver, brain, heart and kidneys (Kassab et al., 2003). Lower serum magnesium concentration was found in one study while other study found normal magnesium concentration (Al-Samarrai et al., 2008). All the β -thalassemia patients in this study showed serum magnesium concentration higher (2.28 ± 0.74 mg/L) than the control (1.11 ± 0.57 mg/L). Increased serum magnesium level is unclear in this study because other studies suggest low serum magnesium in β -thalassemia patients (Abbasiano et al., 1991). However, our study showed hypermagnesaemia in all patients compared to control which may be due to food habit, treatment and physical built up.

The serum zinc (Zn) level in our study was not changed significantly (2.41 ± 0.34 mg/L) in thalassemia group and control subject (2.24 ± 1.02 mg/L). Our study sharply is contrasting with the Egyptian study report where low level of zinc was observed in β -thalassemia population (Yaghmaie et al., 1994). It may be due to mixed β -thalassemia patients (major, minor and intermediate and HbE) in the sample pool

Fluctuations in the essential elements levels seem to be related to the different complications associated with the disease. Zinc deficiency may be attributed to hyperzincuria resulted from the release of Zn from hemolyzed red cells. Assessment of serum ferritin, three major trace elements and hemoglobin profile in patients with beta thalassemia revealed different levels in different studied population of geographic location suggesting variation in the factors influencing their levels in each patient and could be useful in follow up patients with beta thalassemia.

Conclusion

We observed significantly higher serum iron concentration along with high ferritin content and higher concentration of serum magnesium in all β -thalassemia patients compared to control. It was reported that hypozincemia is common in β -thalassemia but our study showed no significant difference of serum zinc concentration. These inconclusive results compared to other countries require more extensive study on micronutrients in β -thalassemia patients of Bangladesh.

Competing Interest: The authors declare that they have no competing interests.

Contribution of Authors: GNNS conceived the idea and drafted the manuscript. HAS analyzed all the samples. RB collected samples and composed Tables. All authors have read and approved the final version of the manuscript.

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Table 1: Patients history from Hospital

| Parameter | Control (n = 48) | β -Thalassemia (n = 110) |
|------------------------------|------------------|--------------------------------|
| Age (years) | 16.21 \pm 1.18 | 12.54 \pm 0.81 |
| Hemoglobin (g/dL) | 11.77 \pm 0.93 | 7.86 \pm 0.36 |
| Blood Hematocrit | 38.90 \pm 3.31 | 28.63 \pm 2.67 |
| Serum ferritin (μ g/mL) | 60.18 \pm 2.87 | 385.76 \pm 82.66 |

Table 2: Blood group of β -Thalassemia patients

| Type of Disease | Blood group | | | |
|----------------------------|----------------|----------------|----------------|-----------------|
| | A ⁺ | B ⁺ | O ⁺ | AB ⁺ |
| HbE β -thalassemia | 03 | 73 | 22 | 02 |
| β -thalassemia minor | 02 | 03 | 05 | |
| Percentage (%) | 4.54 | 69.07 | 24.54 | 1.8 |

Table 3: Serum content of iron, magnesium and zinc according to experimental and control group

| Variables | | Mean | | | |
|----------------------|----------------------|------|-------------------|------------|--------|
| ($\mu\text{g/mL}$) | Group | N | (mg/L) | SD | Sig |
| Iron (Fe) | β -thalassemia | 110 | 3.76 | ± 1.65 | <0.001 |
| | Control | 48 | 2.01 | ± 1.13 | |
| Magnesium (Mg) | β -thalassemia | 110 | 2.28 | ± 0.74 | <0.001 |
| | Control | 48 | 1.11 | ± 0.57 | |
| Zinc (Zn) | β -thalassemia | 110 | 2.41 | ± 0.34 | >0.05 |
| | Control | 48 | 2.24 | ± 1.02 | |