Growth Parameters and Vitamin D status in Children with Thalassemia Major in Upper Egypt

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Received: 12, Jul, 2013
Accepted: 24, Jul, 2013

ABSTRACT
Aim: the aim of this study is to assess the growth parameters, vitamin D, calcium, and phosphorous status in children with thalassemia major receiving packed red cells transfusion with chelation therapy.

Patients and Methods: In a case control study, 100 patients with beta thalassemia major (aged from 4 to 15 years) were compared with 100 sex- and age-matched children serves as a control group. Anthropometric measurement, Serum level of calcium, phosphorus and vitamin D (25 hydroxycholecalciferol) were estimated for all patients & controls.

Results: 49% of our patients had short stature. 47% were underweight. BMI of 43 (43%) patients were low. The mean total serum calcium (6.6±1.2 mg/dl) and 25-hydroxycholecalciferol (25-OH Vit D) (10.4±4.6 mcg/dl) levels were significantly lower in our patients than in controls (10.2±1.06 mg/dl and 40.2±12.3 mcg/dl, respectively); each P< 0.001.

Conclusion: Children with beta thalassemia major have delayed growth and metabolic abnormalities that signify the importance of therapeutic interventions. The presence of these abnormalities may be due to iron overload and poor nutritional support.

Key words: Thalassemia major, Calcium, Growth, Vitamin D

INTRODUCTION
Thalassemia is a heterogeneous inherited disorder of hemoglobin synthesis due to mutations of the globin gene, leading to various degrees of quantitative defect in globin production and reduced synthesis or complete absence of one or more of the globin chains, resulting in ineffective erythropoiesis and anemia. Beta-thalassemia major (BTM) is usually presented at 4 - 6 months of life, due to the protective effect of high hemoglobin F concentration at birth that slowly declines through the first year of life. Manifestations are those of anemia (pallor, lethargy...etc), failure to thrive and organomegaly. Patients presenting later will have signs of extramedullary hematopoiesis; (frontal bossing of the skull, hepatosplenomegaly, thinning of long bones cortices, widening of medullary and diploic spaces; resulting in bossing of skull, prominence of the upper incisors and wide separation of orbits).¹ ² BTM is a significant public health problem in Egypt where over 1–5 million newborns are expected to be affected with this disorder and it is considered the most common genetically determined chronic hemolytic anemia (85.1%) in our locality. A high rate of carriers has been reported in Egypt ranging from 4–10%. This is
due to high rate of consanguineous marriage which helps to accumulate deleterious genes in families, reaching 35.3% in our community.\(^3\),\(^4\) Children with untreated or partially treated thalassaemia major die in the first or second decade of life. The mainstay of treatment is based on adequate, safe blood transfusions and prevention of iron overload. The most widely accepted blood transfusion protocol aims to increase the concentration of hemoglobin to 13-14 g/dl after transfusion, and maintain it at 9-10 g/dl at all times, most patients require transfusion between 2-4 weekly commencing at around 1 year of age.\(^1\),\(^5\) On the other hand, frequent blood transfusion in turn may add to iron overload which may result in hemosiderosis, the later may be a cause of hypogonadism, diabetes mellitus, hypoparathyroidism and other endocrine abnormalities.\(^6\) Several studies reported a higher incidence of growth & endocrine abnormalities in children, adolescents and young adults with BTM.\(^7\),\(^8\) Moreover, abnormalities of serum levels of vitamin D, calcium and phosphorous have been reported in such patients.\(^9\),\(^10\)

The paucity of data in our population prompted us to plan this study aiming to assess growth parameters, vitamin D, calcium, and phosphorous status in children with BTM receiving packed red cells transfusion with chelation therapy.

MATERIALS AND METHODS

The study was approved by the Ethical Committee of Assiut University, Assiut, Egypt. An informed written consent in accordance with Assiut University Ethical Committee guidelines was taken from guardians of all cases and controls.

Patients

Our study included 100 patients with BTM (aged from 4 to 15 years) attending Thalassemia center, Children Hospital, Assiut University, randomly selected to participate in this case control study during the period from September 2009 to March 2013. The diagnosis of BTM was based on standard criteria.\(^11\) All patients included in the study were stable with regular packed red cells transfusion every 1-2 months since early childhood to maintain hemoglobin levels above 9 g/dL. All patients had chelating therapy consisting of 30 to 50 mg/Kg deferoxamine, subcutaneously infused 8-12 hours every night; at least five nights a week. Cases with hypersplenism, other chronic hemolytic anemia, feeding difficulties or malnutrition diseases were excluded from the study. Also any patient received steroid therapy was excluded. Hundred healthy children with comparable age and sex were included as a control group. All control cases were in a good nutritional status with no feeding difficulties or signs of malnutrition. Furthermore, all cases and control cases were not receiving Ca, phosphorus and vitamin D containing preparations.

Biochemical measurements

Serum level of calcium was estimated using o-cresolphthalein direct method, serum level of phosphorus was estimated using Fiske and Subbarow method,\(^13\) and serum level of vitamin D (25 hydroxycholecalciferol) was estimated using 25-OH Vit D ELISA Kit; Immunodiagnostik AG, Germany. Normal level of vitamin D is defined as a 25-OH Vit D concentration greater than 30 ng/mL (75 nmol/L). Vitamin D insufficiency is defined as a 25-OH Vit D concentration of 20-30 ng/mL (50-75 nmol/L).
Vitamin D deficiency is defined as a 25-OH Vit D level less than 20 ng/mL (50 nmol/L).

Statistical analysis

Statistical Package for Social Sciences (SPSS) program version 11 was used for data analysis. Data were interpreted as mean ± SD. Comparison between groups was done by using Student’s t-test for quantitative independent variables. P-value of 0.05 or less is considered significant.

RESULTS

Demographic and clinical data

The mean age of the studied thalassemic patients and controls was 7.35 ± 4.7 and 7.87 ± 4.1 years, respectively. The mean body weight and height of our patients [(15±8.8 kg) (96±86 cm)] were significantly lower than that of controls [(26±17 kg) and (119±25 cm)]; P<0.001 for each (Table 1). 49% of our patients were short (height z-score <−2), compared to 2% of control group (P< 0.001). 47% of our patients were underweight; 38 % were moderate (weight z-score <−2) and 9 % were severe (weight z-score <−3). BMI was abnormal in 43% of our patients (BMI <18.5). No significant correlation was found between growth retardation and sex, anemia and transfused iron load.

Biochemical studies

The mean total serum calcium (6.6±1.2 mg/dl) and 25-OH Vit D (10.4±4.6 pg/ml) levels were significantly lower in our patients than in controls (10.2±1.06 mg/dl and 40.2±12.3 pg/ml, respectively.); each P< 0.001 (Table 2). Only 9% of our patients had normal serum 25-OH Vit D concentration, while 37 % had vitamin D deficiency and the rest (54%) had vitamin D insufficiency. Regarding serum phosphorous; there is no significant difference between patients (3.2±0.6 mg/dl) and controls (3.1 ± 0.8 mg/dl). No significant correlation was found between 25-OH Vit D level and age, sex, anemia and transfused iron load.

DISCUSSION

BTM patients are subjected to a variety of complications such as growth impairment, endocrinopathy and metabolic abnormalities.6-8

In our study the mean height of our patients was significantly lower than that of controls (Table 1). 49% of our cases were of short stature (height z-score <−2). This is in agreement with previous reports.14-18 Hashemi et al., found 65.71% of thalassemic patients had height less than fifth percentile.14 Vogiatzi et al., found 25% were of short stature (height z-score < -2).15 Jain et al., reported 28% of patients had height less than fifth percentile.16 Also Shamshirsaz et al.17 and Flynn et al., reported that their thalassemic patients had short stature compared to controls. Chekir et al., stated that their BTM patients had height lateness by 7.14%.19 In contrast; other investigators reported no significant difference in height between cases and controls.20

Regarding body weight and BMI; the mean body weight of our patients was significantly lower than that of controls (Table 1). 47% of patients were underweight also BMI was abnormal in 43%. Our findings were in agreement with previous studies;14,16,17,19 Hashemi et al., reported underweight in 45.71% and low BMI in 18.6% of their patients with BTM.14 Jain et al., found 20% were underweight.16 Shamshirsaz et al.,17 reported a low body weight compared to controls and Chekir et al.,19 reported weight lateness in their patients by 14.28%. However few reports; claimed that the mean body

| Variable                  | Cases     | Controls | Significance |
|---------------------------|-----------|----------|--------------|
| Serum Calcium (mg/dl)     | 6.6 ± 1.2 | 10.2 ± 1.06 | P< 0.001    |
| Serum Phosphorous(mg/dl)  | 3.2 ± 0.6 | 3.1 ± 0.8 | NS          |
| Serum 25 hydroxycholecalciferol (pg/ml) | 10.4 ± 4.6 | 40.2 ± 12.3 | P< 0.001  |
weight and BMI of their thalassemic patients were in the normal range and insignificantly different than their controls.\textsuperscript{15, 20}

Growth failure is common in patients with thalassemia. Our study and the previous reports suggest the incidence of growth deficits ranges from 20\% to 65\% depending on severity of disease. However, growth failure is multifactorial in thalassemia, related to chronic hypoxia due to chronic anemia, chelation toxicity, low serum zinc level, hepatic iron overload with hepatic dysfunction and iron associated endocrinopathies such as hypogonadism, hypothyroidism, and growth hormone deficiency.\textsuperscript{15-19}

The mean serum level of 25-OH Vit D was significantly lower in our thalassemic patients than in controls (Table 2), 37 \% had vitamin D deficiency and 54\% had vitamin D insufficiency. Vogiatzi et al., reported that 12\% of thalassemic patients were vitamin D deficient and 69.8\% had insufficient levels.\textsuperscript{19} Another study on Iranian population found that 37.2\% of thalassemic patients had vitamin D deficiency.\textsuperscript{17}

Low vitamin D concentrations have been reported previously in thalassemia patients by many authors.\textsuperscript{9, 10, 15, 17, 21-23} They attributed their results to hepatic dysfunction which lead to defective hydroxylation of vitamin D and so decreased serum level. A previous study stated that vitamin D (25-OH Vitamin D) is deficient in thalassemic patients especially in winter than in summer due to geographical attitude, air quality, cloud cover, clothing, time of the day, sun screen use.\textsuperscript{10} Others reported that the cause of 25 OH-D deficiency may be due to the iron-overload in the liver rather than the dysfunctions of endocrine tissues.\textsuperscript{25} Finally individuals with thalassemia may be at greater risk for vitamin D deficiency and therefore have a greater need for vitamin D supplementation.

The serum calcium level was significantly lower in our thalassemic patients than that of controls (Table 2). Our results was in agreement with Shamshirsaz et al.,\textsuperscript{17} Zamboni et al.,\textsuperscript{24} Aleem et al.,\textsuperscript{25} and Autio et al.,\textsuperscript{26} who found hypocalcemia in their thalassemic patients. They explained their results by the presence of iron overload and hemosiderosis resulting in endocrinopathies. Chelation therapy in addition to cirrhotic changes due to hemosiderosis may also play a significant role in hypocalcemia.\textsuperscript{20, 28}

On the other hand some authors found no significant difference between the patients and controls in serum calcium level.\textsuperscript{29-31} Contrarily, single report found that serum calcium level in thalassemic patients was significantly higher than that of controls.\textsuperscript{19} Their results were unexpected, and they found no satisfactory explanation.

Regarding phosphorous level; we found that there is no significant difference between patients and controls (Table 2). These results were in agreement with studies reported that phosphorous levels were within the normal range in patients compared to controls.\textsuperscript{29, 30} Other reports found that serum phosphorous levels were significantly higher in thalassemic patients than the control groups.\textsuperscript{21, 32, 33}

In conclusions; children with BTM have delayed growth and metabolic abnormality that signifies the importance of therapeutic interventions. The presence of these abnormalities may be due to iron overload and poor nutritional support.

**Recommendations**

It is important to emphasize that treatment of thalassemia patients with aggressive nutritional support and supplementation with vitamin D and highly recommended. As we expect defective vitamin D hepatic hydroxylation; we think that vitamin D administration to such patients must be in the form of 1\&25 dihydroxycholecalciferol.

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