How Thalassemia Affects Endocrinological, BMD and Bone Metabolism Parameters - A Cross Sectional Study

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Abstract

Introduction: Beta-thalassemia major patients frequently have endocrine and metabolic disorders. As Iranian studies are scarce; we studied thalassemia major effects on glucose metabolism, endocrinological, BMD and bone metabolism parameters in Iranian patients in a cross sectional study.

Materials and methods: Thirty beta-thalassemia major patients with mean age of 18.2 ± 11 (2-45 y/o) entered the study. Females were 8 cases. Children were 18 cases (less than 20 y/o). Medical history collected and serum levels of ferritin, prolactin, LH, FSH, T4, T3, TSH, IGF-1, testosterone (in males) or estradiol (in females) ACTH, cortisol and Bone-specific alkaline phosphatase and osteocalcin (bone formation markers), NTX (bone resorption marker), Ca, P, Alk ph, PTH and Vit D determined. BMD measured by Hologic Discovery QDR model .

Results: Diabetes, impaired fasting glucose (IFG), low IGF1, short stature, subclinical hypothyroidism, hypocortisolism high prolactin level were found in 7%, 10%, 63%, 40%, 10%, 10% and 6.6% of our patients, respectively. Low Vit-D and Z-score ≤ -2 in spinal and femoral regions (neck and total) found in 76%, 43%, 20% and 16% of our patients. Women showed significantly higher FSH (p-value=0.048). Ferritin related only with prolactin and LH (negatively) and positively with Phosphorous (p-values: 0.027, 0.049 and 0.016 respectively). Age related positively to height, weight, BMD of femoral (neck and total) and spinal regions (p-values<0.001, <0.001, 0.005, 0.013 and <0.001, respectively). T3, total alkaline phosphatase, bone specific alkaline phosphatase, NTX and Z-scores of total femoral and spinal regions related negatively with age (p-values, 0.006, 0.041, 0.008, 0.015 and <0.001, respectively). Mean of ferritin level was significantly higher in diabetic patients (p-values <0.00). Mean age of patients with diabetes, IFG, short stature patients and who had Z-score less than -2 in spinal region were significantly higher than those who were normal (p-values: 0.024 and 0.002 respectively).

Conclusion: The main factor related with diabetes, IFG short stature and lower Z-scores of the femur and spine was age. So, we recommend early monitoring of thalassemia patients (in their childhood) for these complications.

Keywords: Diabetes; Bone-Mineral Density; Thalassemia; Paediatrics; Endocrine disorders

Abbreviations: SD: Standard Deviation; Hb: Hemoglobin; FBS: Fasting Blood Sugar; NTX: N-Terminal Telopeptide; BMD: Bone Mineral Density

Introduction

Thalassemias are forms of inherited autosomal recessive blood disorders which are the most prevalent monogenic disease around the world, among them, beta-thalassemia is characterized by reduced or absent synthesis of beta-globin chains [1] In the homozygous state of beta thalassemia (thalassemia major), which usually is detected during the first two years of life, anaemia is so severe that life cannot be supported without regular blood transfusions. Complications can be caused by frequent blood transfusion and iron overload. Increased haemolysis of transfused erythrocytes

results in excess of hemosiderin, the iron-containing pigment from the breakdown of haemoglobin, and its deposition in various tissues. Complications include heart disease (cardiomyopathy), chronic liver hepatitis, endocrine problems (hypogonadism, hypothyroidism, diabetes, hypoparathyroidism), stunted growth and osteoporosis. Perera and colleagues showed an association between longer duration of transfusion therapy and more endocrinopathies [2].

Growth failure is one of the most common endocrinopathies in transfusion-dependent thalassemia [3,4]. A study performed by Soliman and colleagues showed that after the age of 4 years, rates of longitudinal growth becomes lower than normal controls and bone age has a delay after the age of 6-7 years. Lower rate of growth velocity and a reduced or absent pubertal growth spurt are the major causes of delayed growth [5]. Decreased IGF-1 secretion occurs in the majority of the thalassemic patients and is mainly due to chronic anemia and hypoxia, chronic liver disease, iron overload and endocrinopathies. One study showed that 50% of enrolled patients had insulin-like growth factor I (IGF-1) levels ≤ 2 SDs below normative values for healthy subjects matched for age and sex [6]. Results of IGF- I levels measurement in short stature patients showed a reduced IGF- I levels in 42% of thalassemic patients [7].

The most common endocrinopathies in Thalassemic patients is Hypogonadotropic hypogonadism with 42% prevalence [8]. Both genders of beta-thalassemia major patients have a delay in primary and secondary sexual puberty [9]. Thyroid dysfunction has been reported with varied degree and prevalence in thalassemia patients. For example Najafipour and colleagues showed a 16% prevalence of hypothyroidism in their thallasemia patients while the prevalence of hypothyroidism reported by other authors was in the range of 13-60% . Authors concluded that their patients may suffer from more subclinical forms of hypothyroidism [3]. It should be noted that thyroid dysfunction and levothyroxine consumption could aggravate the risk of bone mineral loss [10,11].

Primary thyroid damage (from iron infiltration) or secondary problems (because of pituitary dysfunction due to hemosiderosis of thyrotrophic cells) are reported in thalassemic patient. Period of transfusion therapy is the most effective factor in the progression of hypothyroidism [2].

Several studies reported a significant prevalence of adrenal dysfunction assessed by an abnormal response to intravenous injection of Cosyntropin [12]. A prevalence of 15.5% of adrenal insufficiency has been reported among thalassemia patients [13]. The range of Vitamin D deficiency and insufficiency is widely varied in thalassemic patients in different countries [14]. One Iranian study reported a very high prevalence of 70% of vitamin D deficiency. Progressive iron overload in liver, that results in 1-hydroxy vitamin D deficiency, and also abnormality in vitamin D absorption in more senile patients are among the causes of vitamin D deficiency [15]. It has been reported that about 40 to 50% of patients with beta-thalassemia major are suffering from osteopenia and/or osteoporosis and even in 30 to 50% of these patients with adequate transfusion and iron chelation, thalassemia-induced osteoporosis have been seen. Therefore, osteoporosis can be considered as a dangerous complication since it can increase the risk of pathologic fracture [16,17].

Although iron overload has been proposed as the main cause of endocrinopathies and growth failure in thalassemia patients, but other factors including low oxygen supply, Desferrioxamine toxicity, cardiac overload, nutritional deficiencies, impaired calcium homeostasis and liver and pancreas involvement may have also a role in this issue [5,18-20]. As it was mentioned, there are different and contradictory data about endocrinological problems in thalassemic patients. The mechanisms of many of these disorders are still unclear. In this cross-sectional study we tried to assess the influence of thalassemia on glucose metabolism and endocrinological and BMD parameters.

Materials and Methods

We recruited 30 known major beta-thalassemia patients, diagnosed by the clinical history and requirement for regular blood transfusion, with no age limit into this study. A medical history was obtained by physicians. The study protocol was approved by the ethics committee of the Endocrinology and Metabolism Research Institute of Tehran University of Medical Sciences and patients signed their consent forms before enrolling into the study.

A stadiometer was used to measure height and weight. Hormonal and biochemical evaluation of study participants was performed by acquiring a fasting blood sample between 08.00 and 09.00 A.M. measuring the following parameters: Hb, FBS, sodium, potassium, calcium, phosphorus, Bone-specific alkaline phosphatase and osteocalcin (bone formation markers), NTX (bone resorption marker), Alk-Ph, PTH, Vitamin D, serum gonadotropins, testosterone(male), estradiol (females), luteinizing hormone (LH), follicle-stimulating hormone (FSH), Cortisol, Prolactin, T4, T3, thyroid-stimulating hormone (TSH), T3Ru, IGF-1. All patients tested by Cosyntropin test.

Study participants underwent a measurement of BMD by Hologic Discovery QDR model. Tanner's pubertal staging was performed based on breast/testicular development and Tanner's staging for pubic/axillary hair. A clonidine stimulation of growth hormone test was performed on patients whose height fall on SD ≤ 3 on growth chart.

Procedures

Serum samples from each participant analyzed at a central facility (endocrinology and metabolism research center laboratories, Tehran, Iran). IGF1 measured by ELISA (IDS, UK) and Ferritin, T3, T4, TSH, LH, FSH, Testosterone, Estradiol, Prolactin, Cortisol were determined by a ELISA (Monobind, USA). For measurement of serum ACTH, IBL (Germany) kits used.

Clonidine stimulation test

Patient should be fast overnight. At the beginning of the test, an intravenous cannula was inserted into cubital vein and fasting sample obtained. Patients took a single oral dose of Clonidine, 4μg/kg. Blood samples were withdrawn every 30 min for GH levels, at 30, 60, 90 and 120 min. Patient checked for the principle adverse effects of Clonidine as dry mouth, dizziness, hypotension (low blood pressure) and drowsiness.

Cosyntropin test

Cortisol and ACTH levels are drawn at baseline (time=0). After that Patients took a single IV dose of synthetic ACTH, The venous blood collected at 30 and 60 minutes and cortisol levels were checked.

Diagnosis criteria for endocrine disorders:

- **Impaired fasting glucose:** Fasting blood glucose >100 mg/dl.
- **Diabetes mellitus:** Fasting blood glucose ≥ 126 or taking diabetes medication.
- **Delayed puberty:** No secondary sexual maturation or any sign of puberty by the age of 12 years in girls and 14 years in boys [21].
- **Hypogonadotropic hypogonadism:** Impaired secretion of gonadotropins, including FSH, LH and testosterone [22].
- **Short stature:** Height that is three standard deviations below the mean height for age and sex (z score ≤ 3).
- **Growth hormone deficiency:** Short stature patients who had at least one positive sample in clonidine stimulation test (positive sample: Growth hormone lower than 10 mg/dl in one of the sampling for growth hormone levels that is carried out every 30 min for 120 min after a single oral dose of 4 μg/kg of clonidine) [23].
- **Insulin-like growth factor 1 (IGF-1) deficiency:** Low levels of IGF1 relative to age and sex specific laboratory norms.
- Sub clinical hypothyroidism: TSH level between 5-10 mlu/ml [24].
- **High prolactin levels:** High levels of prolactin relative to age and sex specific norms, hypocortisolism defined as serum cortisol less than 18 μg/dl at 30 and/or 60 minutes after the synthetic ACTH injection [25].
- **Vitamin D deficiency:** 25(OH)D level less than 20 ng/ml (50 nmol/l) according to laboratory norms.
- **Low bone mass:** Z scores ≤ -2 BMD Z relative to age and sex specific norms.

Results

A total of 30 individuals were enrolled into the study from which 8 patients were female (26.7%). Mean age of the participants was 18.2 ± 11 years (range 2-45 years) while 18 of patients aged less than 20 years (paediatric patients). The mean age of start of oral iron-chelating was 4.3 ± 2.8 years. Most of the patients were diagnosis at the first two years of life with blood transfusion being started at that time for them. Mean height of participants was 142 ± 2.24 cm and their mean weight was 38.2 ± 1.4 kg. Mean of hormonal results showed in **Table 1**. Impaired fasting glucose was found in 3 patients, diabetes in 2 patients, delayed puberty in 2 patients, hypogonadotropic hypogonadism in 4 patients, growth hormone deficiency in 5 patients, IGF-1 deficiency in 19 patients, short stature in 12 patients, sub clinical hypothyroidism in 3 patients, high prolactin levels in 2 patients and Vitamin D deficiency in 23 patients. Low bone density of spine, femoral neck and femur total were seen in 13, 6 and 5 of patients respectively. The mean age in patients who had impaired fasting glucose (p=0.016), diabetes (p=0.003), z score ≤ -2 (p=0.002) and were short stature (p=0.024) was significantly higher.

Table 1 Hormonal characteristic of patients.

Hormones	Results	
	Mean	**Standard Deviation**
*T3 (ng/dl)	146.8	± 27.32
*T4 (μg/dl)	7.7	± 1.2
*TSH (mlu/ml)	2.8	± 1.6
*T3Ru (%)	27.6	± 2.3
*LH (mlu/ml)	2.5	± 2.9
*FSH (mlu/ml)	2.7	± 1.9
Prolactin (ng/dl)	12.3	± 11.8
IGF1 (microg/lit)	77.3	± 64.3
Cortisol (μg/dl)	12.2	± 4.7
*In patients under treatment, hormone therapy not disconnected		

Serum levels of FSH were significantly higher in female subjects (p=0.048). Diabetic subjects had remarkably higher levels of ferritin (p<0.001) compared to non-diabetic subjects. There was a negative correlation between ferritin levels and prolactin (p=0.027) and LH (p=0.049) and a positive relation between ferritin and phosphorous (p=0.016). Age of participants showed a positive association with following parameters: height (p<0.001), weight (p<0.001), BMD of spine (p=0.013) and BMD of femur (p<0.001).

However a reverse association was presented between age and T3 (<0.001), Alk p (p=0.006), bone alkaline phosphatase (p=0.041), NTX (p=0.01), spine z score (p=0.008) and femur z score (p=0.015).

Discussion

In this study we have shown a high prevalence of disturbed glucose metabolism, endocrinopathies and low bone density among our thalassemic patients in comparison to non-thalassemic population [26-34] **(Table 2)**. It should be noted

that this high prevalence of complications seen in our study, is despite the young age of our patients (18.2 ± 11 years).

Table 2 Comparison of Endocrine disorders in thalassemia major with non-thalassemics population.

Endocrine disorders	Prevalence	
	In non-thalassemic Population	In our thalassemic patients
Subclinical hypothyroisam	8.7% (26)	*10%
Diabetes	7.4% (27)	7%
Impaired fasting glocuse	14.6% (28)	10%
Hypogonadothropic Hypogonadism	1/4000 (29)	*18%
Short Stature (in children)	12.4% (30)	27%
GH deficiency	1/4000 (31)	17%
Low IGF1 in short population	20% (32)	100%
Hyperprolactinemia	0.4% (33)	6.6%
Secondary Hypocotisolism	280/1,000,000 (34)	10%
*In patients under treatment, hormone therapy not disconnected		

We observed Vitamin D deficiency in 76%, spinal Z-score ≤ 2 in 43%, femoral head z score ≤ 2 in 20% and femur z score ≤ 2 in 16% of our patients. Impaired fasting glucose was found in 10%, diabetes in 7%, IGF-1deficiency in 63%, short stature in 40%, sub clinical hypothyroidism in 10%, and hypocortisolism in 10% of thalassemic patients. In another Iranian study, prevalence of diabetes was 5% and in other countries it is reported in 12.5-27% [35-37].

Short stature found also reported 26.1%-70% [35,36]. Low IGF1 and GH deficiency and hypocortisolism found in 50% [6], 20% to 50% [37,38] and 32% to 61% [35,39], respectively in patients of other countries. Low BMD reported in 30%-50% [17,39].

The favorable endocrine status in many of endocrine axes of our patients in comparison to other countries might be the result of successful progress towards the implementation of an active 'health network service' that was formed in response to a large population of thalassemic patients in Iran. However may be low sample size is the reason of low prevalence of endocrine disorders in our study

Mean age of our patients with short stature was significantly higher. Older age can positively affect the mineral density of spinal while produces a negative effect on z scores of spine and femur. In other words, older patients have higher absolute value of BMD while in comparison to age and sex specific norms; they have lower levels of BMD.

Although we could not define an association between age and serum levels of TSH, prolactin, Vitamin D, FSH and LH, but older patients had higher prevalence of low IGF-1, (75% vs. 55%) hyperprolactinemia (16% vs. 0%) and lower prevalence of hypothyroidism (3.8% vs. 11.1%), hypogonadotropic hypogonadism (0% vs. 22%) and Vitamin D deficiency (16.7% vs. 72%).

Lower prevalence of hypothyroidism, hypogonadotropic hypogonadism among our older patients might be caused by prescribed HRT, T4, Calcium and vitamin D to older patients. However due to nature of our study we could not exclude the older patients, also we could not recommend our patients to not consume the supplement and this may cause difficulty in interpreting our data. Despite the observation that HRT consumption in older patients decreased the hypogonadism risk but it could not affect the short stature prevalence which might be caused by a delay in diagnosis or treatment start. Female subjects had a higher prevalence of hyperprolactinemia (12% vs. 4.5%) and hypothyroidism (12.5% vs. 9%). While risk of IGF-1 deficiency (50% vs. 68%) Vitamin D deficiency (25% vs. 77%) and Hypogonadotropic hypogonadism (0% vs. 18%) was lower in women. We have no scientific explanation for such gender specific prevalence differences.

There was no association between many of hormone and BMD parameter and iron overload and its management (ferritin and age of start of chelating therapy). As we know ferritin level is not a very good criterion for iron overload and maybe due to the lack of precise criteria, no relation found [18]. May be performing other measurements of evaluations for iron overload such as superconductive quantum interference device (SQUID), or MRI or liver biopsy, improved the validity of our findings

However Perera and colleagues recommended that alterations related to puberty should be placed under surveillance from the age 10 to 12 years. This includes assessment of primary or secondary sexual characteristics development and endocrine examinations in patients suspected of delayed puberty. In addition, in adults patients the regular clinical review and annual monitoring of gonadotropins and sex hormone levels is highly recommended [2].

Conclusion

In this study we found higher prevalence of short stature and hypogonadism in older patients, but even our older patients were not very old, therefore we recommend the early monitoring (late childhood) of endocrine system and BMD in thalassemic patients. The cross-sectional design of this study produces a limiting factor in order to be able to find an association between major independent factors and endocrine function. Prospective studies with bigger sample size are recommended. Moreover, comparing the results with age and sex matched control in case control studies might be helpful.

Ethical Approval

The study protocol was approved by the ethics committee of the Endocrinology and Metabolism Research Institute of Tehran University of Medical Sciences and patients signed their consent forms before enrolling into the study.

Availability of Data and Materials

The datasets during and/or analysed during the current study available from the corresponding author on reasonable request.

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Author's Contributions

Study design: Fariba Mohseni, Amir Ali Hamidieh, Mohammad Reza Mohajeri-Tehrani, Bagher Larijani, Ardeshir Ghavamzade, Kamran Alimoghaddam, Zohreh Hamidi. Study conduct: Amir Ali Hamidieh, Maryam Behfar Fariba Mohseni, Zohreh Hamidi. Data collection: Fariba Mohseni, Zohreh Hamidi, Ameneh Naghghash, Sarah Shirazi Data analysis: Zohreh Hamidi, Hoda Rashidian. Data interpretation: Mohammad Reza Mohajeri-Tehrani, Fariba Mohseni, Zohreh Hamidi. Drafting manuscript: Zohreh Hamidi, Maryam Aboee-Rad Revising manuscript content: Mohammad Reza Mohajeri-Tehrani. Approving final version of manuscript: Mohammad Reza Mohajeri-Tehrani. Zohreh Hamidi takes responsibility for the integrity of the data analysis.

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