Original Article

Endocrine and metabolic disorders in β-thalassemia major patients

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Received: 7 April 2012
Revised: 8 May 2012
Accepted: 15 May 2012

Abstract

Background: Thalassemia is the most common hereditary anemia and beta thalassemia major is its most severe form. Endocrine abnormalities in thalassemia major are common disturbing complications that need prompt management. The purpose of this study was to determine the endocrine disorders and bone mineral density in patients with major β-thalassemia in Qazvin, Iran**.**

Methods: In this cross- sectional study, 77 patients with β- thalassemia major (15-36 years old) were enrolled. Physical examination, laboratory tests, bone radiography and bone density measurements were performed. Then, the data were analyzed.

Results: Forty patients were males. The mean age was 21.26±4.53 years old. The mean BMI was 20.15±2.79 kg/m^2. Impaired puberty, short stature, hypothyroidism, diabetes mellitus, IGT, hypoparathyroidism, vitamin D deficiency and vitamin D insufficiency were observed in 46.8%, 33.8%, 18.2%, 16.9%, 13%, 7.8%, 45.5% and 24.7% of patients, respectively. Nearly 80% of patients had low bone mineral density. Bone mineral density was significantly associated with hypogonadism (p=0.001), short stature (p=0.026), hypoparathyroidism (p=0.031), hypothyroidism (p=0.048), diabetes mellitus (p=0.002) and vitamin D deficiency (p<0.001).

Conclusion: Impaired puberty and short stature were the most common endocrine complications in our population. Low bone density (osteopenia, osteoporosis) is significantly different in β-thalassemic patients with and without endocrine complications.

Keywords: Major β- thalassemia, Bone mineral density, Osteopenia, Osteoporosis, Puberty

Caspian J Intern Med 2012; 3(3): 466-472

Thalassemia is heritable disease causing unbalanced globin chain synthesis ineffective erythropoiesis and increased peripheral hemolysis (1-3). Hemoglobinopathies such as major β-thalassemia used to be a potentially lethal disorder in childhood, but optimized transfusion programs and chelation therapy (introduce since the 1970s) had improved patients' life expectancy and quality (4). But now the patients are experiencing a new range of problems, particularly in relation to their growth and development (5). Thalassemia patients show a variety of bone disorders including bone pain or deformity, bone age delay, growth failure, rickets, scoliosis, spinal deformities, nerve compression, pathologic fracture, osteopenia or osteoporosis (5-7). Osteoporosis is the most prevalent bone complication in β-thalassemic patients despite regular transfusions and iron chelation therapy (8). This supposed to determine the endocrine disorders and bone mineral density in patients with major β-thalassemia referred to thalassemia center in Qazvin, Iran**.** Also it is supposed that low bone mass in patients with thalassemia is more of a reflection of endocrine abnor`malities rather than hematological problems (2).

For instance, adolescents with thalassemia frequently have delayed puberty (3). The lack of growth and sex hormones in the polytransfused subjects lead to a subsequent failure of bone mineral accretion (1).

Majority of the previous researches have studied the adults' population of the Mediterranean origin and there are few studies on children and young adults from the Asian people (5). Previous studies on thalassemic patients in Qazvin were absent so we designed this study to determine the endocrine disorders and bone mineral density in patients with major beta thalassemia referred to Thalassemia Center in Qazvin, Iran.

Methods

The subjects were all patients with known β-thalassemia major (TM) with hemoglobin electrophoresis and clinical manifestations who had attended Hematology and Endocrinology Clinics Children's Hospital, in Qazvin province, Iran. Qazvin is a city located 150 kms from the capital city of Iran, Tehran. The diagnosis of thalassemia intermedia was exclusion criteria of the study.

The study was approved by the Human Research Ethics Committee of Qazvin University. The subjects completed a consent form. All patients were under regular transfusion program (every 14-100 days) with the aim of maintaining pre-transfusion hemoglobin (Hb) levels above 9 g/dl. All patients received subcutaneous desferrioxamine, folic acid and multivitamin supplements. An informed consent was obtained from the participants, their parents or legal guardians. Physical examination was performed by one pediatric endocrinologist who recorded the patients' height, weight and sexual maturation scores (Tanner staging). The height was measured using a metal stadiometer to the nearest 1 mm. The body weight was measured with accuracy of 100 gram by using Seca Scale (made in Germany) while subjects were lightly dressed. Height and weight percentiles were compared with NCHS (National Center for Health Statistics) growth charts. The body mass index (BMI) was calculated by dividing the weight in kilogram by the square of height in meters. The short stature was defined as height less than -2 standard deviation (SD) of the age- and sex- matched population (9).

Pubertal stages were determined by both visual inspection and palpation, using the criteria and definitions described by Marshal and Tanner (10, 11). Impaired puberty in TM patients include delayed puberty, arrested puberty and hypogonadism. Delayed puberty was defined as the absence of breast enlargement in girls and testicular enlargement in boys by the age of 13 and 14 years respectively. Arrested puberty is defined as the absence of pubertal progression for more than one year after puberty onset, where testicular volume in boys is less than 6 to 8 ml and unchanged breast size in girls. Puberty onset delay more than 2 SD beyond the mean for sex was considered as hypogonadism (10-12). The bone maturation was assessed by the left hand wrist x-ray and average bone age (BA) was calculated by using Greulich and Pyle method by the endocrinologist who was blind to the age and Tanner stages.

Bone mineral density (BMD) was done for all the subjects. BMD included anteroposterior lumbar spine (L1-L4) and femoral neck scanning were done using dual energy x-ray absorptiometry, (It was daily calibrated according to the manufacturer's instructions). The standard deviation (SD) between BMD and the expected healthy age matched population was expressed as T-score. Osteopenia was defined as T-score between -1 to -2.5, and T-score below -2.5 was considered as osteoporosis by WHO criteria (13).

Blood samples were obtained to determine serum calcium (Ca), phosphate (P), alkaline phosphatase (ALP), fasting blood sugar (FBS), ferritin, intact parathyroid hormone (iPTH), free T4 (FT4) ,thyroid stimulating hormone(TSH), follicle stimulating hormone (FSH), luteinizing hormone (LH), testosterone for boys and estradiol for girls.

Fasting blood sugar (FBS) was measured by SELECTRA E analyzer using the photometric assay method and reagent purchased Parsazmun Company, IRAN; Mean intra- and interassay coefficients of variation (CVs) were 1.28% and 0.84%, respectively. The oral glucose tolerance test (OGTT) was performed by 75gr glucose in fasting patients. Impaired glucose tolerance (IGT) was defined as a glucose level greater than 140 mg /dl; but, less than 200 mg / dl at two hours. Diabetes mellitus was defined as OGTT ≥200 mg/dl or based on the history of insulin therapy (2). Ferritin was measured by the two-site immunoradiometric assay (IRMA), using reagent purchased from Pishtaz Teb Company, Iran. Mean intra- and inter-assay CVs were 6.3% and 7.1%. PTH was measured by the two-site immunoradiometric assay (IRMA), using reagent purchased from Roche Company, Germany. Mean intra- and inter-assay CVs were 3.6% and 3.9%. FSH and LH levels were

analyzed by the two-site immunoradiometric assay (IRMA), using reagent purchased from Monobind Company, USA. Mean intra- and inter-assay CVs were 3.3%, 5.3% and 4.3%, 4.2%, respectively. Testosterone was measured by radioimmunoassay (RIA) technique using reagent purchased from Monobind Company, USA. Mean intra- and inter-assay CVs were 3.3% and 6.7%. Estradiol was measured by fluorescent assay (ELFA) technique using reagent purchased from IBL Company, Germany. Mean intra- and inter-assay CVs were 2.71% and 6.7%. FT4 was measured by radioimmunoassay (RIA) technique using reagent purchased from Diaplus Company, USA. Mean intra- and inter-assay CVs were 4.14% and 7.2%. TSH level was measured by radioimmunoassay (RIA) technique using reagent purchased from Monobind Company, Germany. Mean intra- and inter-assay CVs were 4 % and 4.2%. Hypothyroidism was recognized as reduced FT4 and increased TSH levels or based on treatment with levothyroxine based on previous diagnosis (14).

25- Hydroxyvitamin (25 OHD) was measured by radioimmunoassay (RIA) technique using reagent purchased from Ids Company, Italy. Mean intra- and inter-assay CVs were 5.3% and 4.6%. 25 (OH) D values less than 10 ng/ml was considered as deficiency and value less than 30 ng/ml classified as insufficiency (15).

Serum Ca concentration was measured by CPC technique using reagent purchased from Parsazemoon Company, Iran. Mean intra- and inter-assay CVs were 0.62% and 2.4%. Serum P concentration was measured by phosphomolybdate UV technique using reagent purchased from Parsazemoon Company, Iran. Mean intra- and inter-assay CVs were 1.12% and 1.31%. Serum ALP concentration was measured by enzyme immunoassay technique using reagent purchased from Parsazemoon Company, Iran. Mean intra- and inter-assay CVs were 0.92% and 0.85%. Hypoparathyroidism defined as low serum PTH (with a normal range of 15-65pg/ml), reduced Ca and increased P. FSH, LH, testosterone, estradiol, TSH, FT4 and bone metabolism parameters were categorized as normal, high or low for their respective chronological ages.

The data were analyzed using SPSS software. Correlation among variables was assessed using chi-square and t-test. The patients experiencing severe low bone mass were categorized in subgroups and comparisons were done among the subgroups by using chi-square of fisher's exact (where small frequencies were involved) test. A p-value less than 0.05 ($p<0.05$) was considered significant.

Results

Seventy seven patients (15–36 years old) with β-thalassaemia major were included in the study. The mean age was 21.26±4.53 years old. Forty patients were males. The characteristics of the patients are shown in table1.

Table 1. Findings of physical examination

Variables	Mean±SD	Minimum	Maximum	95% CI
Weight (kg)	50.50± 8.91	38.5	69	48.48-52.53
Height (cm)	159±10.03	136.7	180	156.73-161.28
BMI (kg/m2)	20.15±2.79	15.80	30.50	19.52-20.79
Transfusion interval (day)	26.42±11.16	14	42.5	23.89-28.96
Duration of transfusion (year)	18.66±4.71	2.5	29	17.59-19.73

Hypogonadism was the most common endocrine complication in 36 (46.8%) patients, 28 (36.4%) with delayed puberty and 8 (10.4%) with arrested puberty associated. Hypoparathyroidism was found in 6 (7.79%) patients and hypothyroidism in 14 (18.18%) of total. 13 (16.88%) patients had diabetes mellitus and 10 (13%) had IGT, 23 patients (29.8%) had hyperglycemia .28 (33.8%) patients had short stature. Other endocrine disturbances are shown in table 2.

Table 2. Distribution of laboratory tests in b- thalassemia patients

Results of laboratory tests	Frequency (%)
Hypocalcemia	6 (7.79)
Hypomagnesaemia	0 (0)
Hyperphosphatemia	14 (18.18)
Increased ALP	33 (42.9)
Vit D deficiency	35 (45.5)
Vit D insufficiency	19 (24.7)

Thirty nine (50.6%) of subjects had osteopenia and 21 (27.3%) had osteoporosis in lumbar spine and 33 (42.09%) had osteopenia and 19 (24.7%) had osteoporosis in femoral neck. The mean Z-score was -2.16±3.62 (-4.66 to 1.65) in lumbar spine and -1.42±1.23 (-3.28 to 1.25) in femoral neck scans. The mean T-score was -1.78±1.06 (-4.44 to 1.60) in lumbar spine and -1.30±1.32 (-3.8 to 1.47) in femoral neck scans. Impaired puberty had significant association with bone mineral density in lumbar spine and femoral neck (p=0.034 and p=0.016, respectively); bone density significantly associated with hypogonadism (p=0.003), hypoparathyroidism (p=0.031), short stature (p=0.026), hypothyroidism (p=0.048) and diabetes mellitus (p=0.001).

The mean bone age was 16.37±2.36 years old in total. There was a significant association between bone mineral density and bone age (p=0.001). There was significant association between vitamin D deficiency and insufficiency with bone mineral density (p=0.001). Impaired puberty, short stature, hypothyroidism, diabetes mellitus, IGT and hypoparathyroidism observed in 46.75%, 33.8%, 18.18%, 16.88%, 13% and 7.79% of patients, respectively. Nearly 80% of patients had low bone mineral density.

Discussion

The life expectancy in patients with TM has increased due to therapeutically management, such as frequent transfusion, desferal administration and bone marrow transplantation. Various complications like several endocrinopathies, cardiomyopathies and bone disorders are common in these patients (16). Low bone density remained as a significant problem (17).

Our survey showed that more than half of the TM patients had osteopenia or osteoporosis. There was a significant correlation between bone mineral density and pubertal status. There are different reports of complications' prevalence that can be due to various methods used in the patient's management, ethnic or individual differences (18).

With multiple transfusions, excess iron appears in the plasma that can cause progressive tissue damage in the liver, heart, endocrine glands, and other organs by generating hydroxyl free radicals, which also causes oxidative stress (19, 20). The human body has a limited capacity to control iron overload. Iron overload is frequently associated with transfusion therapy in patients with thalassemia major (19, 21). The accumulation of iron in various organs may result in known complications, including diabetes, hypogonadism, hypothyroidism, low bone mass and hypoparathyroidism (21). Previous researches showed a high prevalence of endocrine abnormalities in thalassemic patients. Iron chelation therapy is the only method of iron overload control in transfusion dependent patients. Despite the use of iron chelation therapy, the pituitary gland, peripheral endocrine tissues and gonad axis are susceptible to iron deposition and damage.

The delay of growth and puberty with reduction of final height happens frequently in this population. Sexual complications such as delayed or arrested puberty and hypogonadism are the most common endocrine complications. Arrested puberty is defined as secondary amenorrhea in females and decline in sexual activity and azzoospermia in males. Iron deposition on the pituitary gonadotrophic cells which is followed by disruption of gonadotrophin production is the considerable reason of hypogonadotrophic hypogonadism. Secondary hypogonadism becomes evident later in life (12).

In Arcispedale et al.'s study, short stature was present in 31.1% of males and 30.5% of females. Delayed puberty was the most common endocrine complication (40.5%) followed by hypoparathyroidism (6.9%), impaired glucose tolerance (6.5%), primary hypothyroidism (3.2%) and insulin-dependent diabetes mellitus (3.2%) (22).

An Italian working group reported delayed puberty in 47% of females and 51% of males, arrested puberty in 12.6% of females and 15.7% of males, and secondary amenorrhea in 25% of adult females (23). Delayed or arrested puberty were also the most prevalent endocrine disorder in our study, which were followed by short stature (33.8%), diabetes mellitus (16.88%), IGT (13%), hypothyroidism (15.9%) and hypoparathyroidism (6.3%).

Delayed growth compare to age may reduce peak bone mass density (24). The etiology of short stature is multifactorial for example: chronic anemia, hypoxia, chronic liver disease, zinc and folic acid deficiency, iron overload, intensive use of chelating agents, emotional factors, endocrinepathies (25). In Shamshirsaz et al.'s study, the prevalence of short stature was 39.3% (26).

The prevalence of diabetes was considerably lower in Italian patients compared with our data (16.88%) (22). The erum ferritin level, genetic factors or other unknown conditions may play the role in the genesis of insulin resistance (27).

Thyroid dysfunction has been reported in 13 to 60 percent of thalassemia patients (28). However, the overall prevalence of hypothyroidism is low (18, 29, 30). It seems that these differences can be explained by therapeutically protocols (blood transfusion and deferoxamine administration) comparing to the patients' age (31). The prevalence of hypoparathyroidism among our patients (6.3%) is comparable with other studies (3.6% to 7.6%) (31, 32).

Bone disorders appear as rickets, scoliosis, severe bone pains, spinal deformity, osteopenia, severe osteoporosis or several fractures. Excess iron deposit in bones may influence osteoblast number and activity and interferes with mineralization, and consequently leads to osteoporosis (3, 33). Nevertheless, there was no correlation between serum ferritin levels and BMD in this study. In present study, a reduction in bone density was also a common problem.

Previous studies have shown a remarkable decrease in BMD values either at both femoral and lumbar (3, 5, 6, 13, 34). Many studies reported that BMD was decreased in all their samples (117 patients with TM) (3, 5, 13, 34). Angastiniotis et al. found 52.5% osteoporosis in the subject's spine using DXA (35). A further group of 30 patients aged between 17 to 44 was scanned using DXA. Their mean spiral BMD z-score was -3.07 (36).

Our findings are generally in agreement with data published by other authors. A study of 82 patients (age mean 25y, range 12-43y) showed that 51% had severely low bone density (BMD z-score < -2.5) and 45% had a reduced bone density (BMD z-score between -1 and -2.5) (37). Voskaridou et al. also reported lumbar and femoral osteoporosis in 50.2% and 11% of their samples (208 patients), respectively (1). Bone density was also lower in 9 to 18 years old Lebanese TM patients (n=29) than the normal matched individuals, and there was correlation with LH, FSH, estrogen and testosterone levels (38). Karimi et al. reported significant correlation between bone density and hemoglobin in patients with TM and intermediate (2). There was no significant difference between genders regarding bone mineral values contrary to other studies groups indicating more bone changes in males than in females (2). Shamshirsaz et al. have similarly showed no significant difference in the prevalence of osteoporosis between boys and girls (26) but, in other studies, thalassemia patients were both more commonly and severely affected with low bone mass (5, 39).

Vitamin D deficiency may start early in TM even before hypoparpthyroidism is established. Vitamin D deficiency potentially contributes to low bone mass in thalassemia. Thalassemic patients progressively develop iron overload and it is possible that a deficiency in liver hydroxylation of vitamin D or in vitamin D absorption appears in older thalassemic patients. However, studies in children (40, 41) and in adult thalassemic patients (42, 43) have shown contradirectory results. Vitamin D deficiency was observed in 13/24 patients in Wood et al.'s study in the USA (44). Vitamin D deficiency and vitamin D insufficiency were found in 35 (45.5%) and 19 (24.7%) patients, respectively. Overall, vitamin D deficiency and insufficiency were seen in 44 (70%) patients. In conclusion, the rate of decreased bone density was 77% in our study, which was only correlated with pubertal status. It did not have any other relation to the other assessed variables. It can be because of hormonal medicine, calcium and the vitamin D prescribed for the patients. Since Iranian thalassemia patients have shown a considerable decrease in bone mass according to our study and the others, annual evaluation of bone mineral status is recommended.

Acknowledgments

We would like to thank Dr Neda Esmilzadeh, the staff of the Center for Clinical Researches at Qazvin Children Hospital (affiliated to Qazvin University of Medical Sciences) and Dr Toktam Karimzadeh for their help in preparing this paper.

Funding: Nothing to declare

Conflict of interest: The authors report no conflicts of interest in this work.

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