Bone-related complications of transfusion-dependent beta thalassemia among children and adolescents

Article *in* Journal of Bone and Mineral Metabolism · March 2013

DOI: 10.1007/s00774-013-0433-1 · Source: PubMed

7 authors, including:

Naghmeh Mirhosseini
The Pure North S'Energy Foundation
58 PUBLICATIONS **301** CITATIONS
SEE PROFILE

Suzana Shahar
Universiti Kebangsaan Malaysia
229 PUBLICATIONS **1,764** CITATIONS
SEE PROFILE

Majid Ghayour-Mobarhan
Mashhad University of Medical Sciences
376 PUBLICATIONS **3,932** CITATIONS
SEE PROFILE

Nor Azmi Kamaruddin
Hospital Universiti Kebangsaan Malaysia (HUKM)
195 PUBLICATIONS **3,465** CITATIONS
SEE PROFILE

J Bone Miner Metab (2013) 31:468–476
DOI 10.1007/s00774-013-0433-1

ORIGINAL ARTICLE

Bone-related complications of transfusion-dependent beta thalassemia among children and adolescents

Naghmeh Zahra Mirhosseini · Suzana Shahar · Majid Ghayour-Mobarhan · Abdullah Banihashem · Nor Azmi Kamaruddin · Mohammad Reza Hatef · Habib Alah Esmaili

Received: 22 September 2012 / Accepted: 28 January 2013 / Published online: 10 March 2013

Abstract Thalassemia and the blood transfusion complications associated with it predispose children to poor bone health. This study was conducted to determine the prevalence of bone-related abnormalities and identify the bone health predictors within this population. One hundred and forty transfusion-dependent beta thalassemic subjects 8–18 years old in Mashhad, Iran, participated in this cross-sectional study. Anthropometric measures, dietary intake, bone-related biomarkers and bone densitometry, were assessed. The incidence of underweight and short stature was 33.6 and 41.4 %, respectively, which were indicators of malnutrition among thalassemic subjects in this study. Low bone density was detected in the lumbar spine and femoral region in 82 and 52 % of subjects, respectively. Hypocalcemia and hypophosphatemia were seen in 22 and 18.2 %, whilst vitamin D deficiency was present in more than 85 % of thalassemic children and adolescents. The relationships between weight, height and other anthropometric indices, serum calcium and bone markers, intake of macronutrients, zinc and vitamin E with bone mineral density (BMD) and bone mineral content (BMC) in the lumbar spine and femoral area were positively related, indicating that better nutritional status were associated with higher BMD and BMC values. Puberty, gender and serum osteocalcin were negative predictors for BMD and BMC values, whereas age, weight and height were the positive predictors. High incidence of low bone density and deficit in other aspects of bone health among thalassemia patients makes routine bone health assessment necessary for this vulnerable group. Considering influencing factors, dietary counseling and preventive supplementation therapy for this high risk group of children and adolescents may be necessary, although this should be assessed by intervention studies.

Keywords Low bone density · Hypocalcemia · Malnutrition · Nutrient intake · Thalassemia

N. Z. Mirhosseini · S. Shahar
Dietetic Programme, Centre for Health Care Sciences, University Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, 50300 Kuala Lumpur, Malaysia

N. Z. Mirhosseini · M. Ghayour-Mobarhan (✉)
Faculty of Medicine, Biochemistry of Nutrition Research Center, Mashhad University of Medical Sciences (MUMS), Azadi Square, Pardis, Mashhad, Iran
e-mail: n.mir@usask.ca

A. Banihashem
Hematology-Oncology Department, Dr. Sheikh Hospital, Mashhad University of Medical Sciences (MUMS), Mashhad, Iran

N. A. Kamaruddin
Internal Medicine Department, Hospital of University Kebangsaan Malaysia, Kuala Lumpur, Malaysia

M. R. Hatef
Internal Medicine Department, Imam Reza Hospital, Mashhad, Iran

H. A. Esmaili
Department of Statistics, Faculty of Medicine, Mashhad University of Medical Sciences (MUMS), Mashhad, Iran

Introduction

Osteoporosis is a global health problem which is increasing in incidence [1]. The identification of modifiable risk factors, especially nutritional factors, which have the potential to improve bone health, will help in the development of preventive strategies [2]. Most effort in bone mass improvement and fracture prevention has focused on

delaying the rate of senile bone loss and reducing the frequency and severity of trauma among the elderly. However, improving peak bone mass early in life is a major determinant of future osteoporosis risk [3, 4]. Bone mass accrual is influenced by heredity, sex, diet, hormones, mechanical forces, and exposure to risk factors [3, 5]. Body weight and consequently food intake is a major determinant of bone density in children and adolescents [5]. The skeleton is a nutrient reserve for calcium and phosphorus. Other dietary components such as magnesium, zinc, copper, iron, fluoride, and vitamins D, A, C, and K are required for normal bone metabolism [6]. For children with chronic disorders, such as thalassemia, identifying ways to increase bone mineral accrual is of particular importance because they have been found to have low bone mineral content (BMC) and bone mineral density (BMD) [7, 8]. Turkish thalassemic children were reported to have higher phosphorous, osteocalcin, serum carboxy terminal telopeptide fragment of type I collagen, intact parathyroid hormone and ferritin levels whilst they have significantly lower 25-hydroxy vitamin D, alkaline phosphatase and z-scores both at lumbar and femur compared to controls in the study of Pirinccioglua et al. [9]. Leung et al. [10] have reported the prevalence of severe bone mineral density deficit in spine and hip respectively as 62 and 35 % among thalassemic patients. Evaluation of osteopathy in Indian transfusion-dependent thalassemic children and adolescents revealed a high prevalence of osteoporosis (81 %), using Dual X-ray Absorptiometry (DXA). High levels of bone resorption and bone formation markers were reported in 55 and 36 %, respectively, in this group. Low serum vitamin D level was seen in 62 % of patients, whilst 38 % had high parathyroid hormone (PTH) levels. This study revealed that the majority of thalassemics with inadequate chelation therapy have increased levels bone resorption with advancing chronological age [11]. Bekheirnia et al. [12] (2004) reported a high prevalence of low bone mineral density (68.7 and 17.6 % at the lumbar and femoral regions, respectively) among 131 transfusion-dependent β thalassemic patients, aged 10–20 years. Potential risk factors for low bone density among Iranian thalassemic children and adolescents include; malnutrition, delayed puberty or hypogonadism, age when iron chelation was started, duration of desferal therapy, and serum zinc concentration [13]. Collectively, the delay in sexual maturation, the presence of diabetes and hypothyroidism, parathyroid gland dysfunction, progressive marrow expansion, iron toxicity on osteoblasts, iron chelation therapy, and deficiency of growth hormone or insulin growth factors and subsequent growth retardation have been identified as major causes of osteoporosis in thalassemia [14]. However, there are relatively little data which considers other aspects affecting bone health that includes; disease history, nutritional, biochemical and dietary intake. This study was conducted to determine the prevalence of low bone density and other complications of thalassemia which are related to bone health, and to identify the factors associated with disturbed bone health among transfusion-dependent beta thalassemic children and adolescents in Mashhad, Iran.

Materials and methods

Ethical approval was obtained from Mashhad University of Medical Sciences Ethic Committee. One hundred and forty transfusion-dependent thalassemic patients in the age range of 8–18 years old, who were registered in a specialized haematology center in Mashhad, were recruited into this study. Thalassemic patients with malabsorption, or other gastrointestinal problems and asthma, patients with diabetes, thyroid disorders or any other diseases, affecting growth and bone health, patients with any history of bone fracture, patients currently taking a bisphosphonate medication for osteopenia or taking any vitamin or mineral supplement, patients who have had a bone marrow transplant and individuals with chronic consumption of systemic corticosteroids and patients with any positive family history of osteopenia or bone fracture were excluded from this study. Informed consent was obtained from parents.

Anthropometric measurements [height, weight, triceps and subscapular skinfold thickness, mid upper arm circumference (MUAC) and waist circumference (WC)] of subjects were taken. Body mass index (BMI) and arm muscle area (AMA) were calculated for each subject. The measurements were taken according to international guidelines [15] by the same person for all subjects. Dietary intake was assessed using a combination of 24-hour diet recall and a 2-days food record. Demographic data, socioeconomic status, the history of disease, initiation and duration of blood transfusion, as well as chelation therapy were included in a face-to-face interview questionnaire.

Bone health was assessed using bone density indices [bone mineral density (BMD) and bone mineral content (BMC) of lumbar spine and femoral area] using a Hologic Digital Absorptionmeter (OSTEOCORE CE0120, France). Bone-related biomarkers [serum calcium, phosphorus, alkaline phosphatase (ALP), parathyroid hormone (PTH), 25(OH) vitamin D, serum osteocalcin and C-telopeptide] were determined. Serum calcium was measured using Calcium Iiquicolor Kit using a photometric test (CPC Method) (Human company, Calcium Iiquicolor, Germany). Serum phosphorus was measured using Quantitative Diagnostic Kit using a photometric Method (UV Test) (ParsAzmun Company, Quantitative Diagnostic, Iran). Serum alkaline phosphatase was measured using DGKC

Method (an Enzymatic-Kinetic Procedure, Centronic GmbH, ALP-FLUID (5 + 1), Germany). Parathyroid Hormone (PTH) was measured using an IRMA PTH Kit via Radioimmunoassay (RIA) in serum (Immunotech, IRMA kit, France). The IDS 25-Hydroxy Vitamin D EIA kit (Immunodiagnosticsystems, EIA kit, UK) was applied for the measurement of serum 25-hydroxy vitamin D (25(OH) D) using an enzyme immunoassay method (Rayto, Japan). Serum osteocalcin was measured using a N-MID Osteocalcin ELISA kit (Immunodiagnostic Systems, UK). C-telopeptide was measured in serum using Serum CrossLaps ELISA kit (Immunodiagnostic Systems, Serum CrossLaps ELISA, UK). Pre-transfusion serum Hemoglobin, fasting blood sugar (FBS), serum ferritin and thyroid function test (TSH, T4) were obtained from the patient's file based on the more recent test results.

The data were analyzed using Statistical Package for the Social Sciences (SPSS), version 16. The mean and standard deviation were used to summarize the variables. Pearson's correlation was tested to determine the relationship between normal variables. For data which were not in normal distribution, Spearman's correlation was performed to show the relationship between variables. Binary logistic regression was applied to specify factors associating with disturbed bone health status. Multiple regressions was conducted to determine the predictors of bone health in study subjects by specifying the confounding factors in this study. Prevalence rate was expressed with 95 % confidence intervals. In this study the statistical significance and power were considered at a P value <0.05 and 80 % respectively.

Results

A total of 140 subjects, (56.4 % male and 43.6 % female), with transfusion-dependent beta thalassemia completed the study. Subjects were divided into preadolescent ($\leq$12 years) (42 %) and adolescent (>12 years) (58 %). Based on the appearance of secondary sexual characteristics, which were reported by parents, 22 % of subjects were in post-pubertal stage. Puberty was attained in 16.5 % of boys and 29.5 % of girls. Iron chelating drug therapy was prescribed for 96 % of the subjects. Desferal was the most common iron chelator which was used regularly, 3–6 times/week, by more than 64 % of subjects. History of bone fracture during childhood, which was more prevalent in boys (31.6 %) than girls (18 %), was reported by 25.7 % of study subjects. Table 1 showed the distribution of age, anthropometric parameters and bone density indices. Malnutrition was detected in 33.6 % of subjects, using the WHO BMI z score charts (2007), which were categorized as underweight, moreover considering short stature, malnutrition was seen in 41.4 % of thalassemic subjects in this study. Underweight and short stature was more prevalent among boys (44.3, 58 %) compared to girls (19.7, 35.5 %). Dietary intake analyses showed that the intake of energy was 57 % of the recommendation (RDA) which was almost half of the subject's requirement. The intake of nutrients, related to bone health, such as calcium, phosphorus vitamin D and zinc was less than RDAs, as shown in Fig. 1.

The distribution of biochemical profile of subjects according to gender and age group is described in Table 2. Hyperglycemia was present in 25.5 % of subjects, more commonly among girls. High TSH level were seen in 11.9 % of subjects, while low level of TSH was seen in 2.5 % of subjects. All the subjects had high levels of serum ferritin. Anemia (Hb $<$ 10 g/dl) was seen in 75.8 % of the sample population. Boys were more anemic than girls. Hypocalcaemia and hypophosphatemia was identified in 22 and 18.2 % of subjects respectively, and more commonly found among pre-adolescents as compared to adolescents. Hypercalcaemia and hyperphosphatemia were present in 10.6 and 41.7 % of the subjects respectively, especially in adolescents. Low serum ALP was found in 7.7 % of subjects. Low PTH level was seen in 3.4 % of subjects. High level of PTH was also present in 3.4 %, more commonly among girls. According to Misra et al. [16] categories of vitamin D deficiency, 84.7 % of subjects had vitamin D deficiency, and 8 % had insufficient vitamin D concentration. This study revealed that more than 90 % of subjects with transfusion-dependent β Thalassemia suffered from some degree of vitamin D deficiency as shown in Table 3. The normative data of healthy Indian subjects, matched for age and gender, was used to determine the percentage of low bone mineral density (BMD) [17]. Based on this categorization, low bone mineral density in lumbar spine was seen in almost 82 % of subjects, while in femoral area 52 % of them had low bone mineral density (Fig. 2). Bone turnover markers showed significant negative correlation with bone density indices (Table 4). The intake of energy and macronutrients, as well as the intake of zinc, iron and Vitamin E were positively correlated with bone mineral density and bone mineral content. The intake of vitamin D was negatively associated with bone mineral density and content (Table 5). Multivariate regression model analysis was undertaken in order to determine the predictors for bone status. In the predictive model for lumbar BMD and BMC, significant positive predictors included age, weight and serum calcium. The significant negative predictors of lumbar BMD and BMC were puberty and subscapular skinfold thickness. Height and age were positive predictors for both BMD and BMC of the femur, while gender and serum osteocalcin were the negative predictors for femoral BMD as was shown in Table 6.

Table 1 Distribution of age, anthropometric characteristics and bone density indices of subjects (Presented as mean ± SD)

Characteristic	Boys ($n = 79$)		Girls ($n = 61$)		Total ($n = 140$) Mean ± SD (range)
	8–12 years ($n = 29$)	13–18 years ($n = 50$)	8–12 years ($n = 30$)	13–18 years ($n = 31$)	
Age (year)	10.00 ± 1.5	16.54 ± 1.8#	9.4 ± 1.5	16 ± 2#	13.5 ± 3.7 (8–18)
Age of diagnosis (months)	9.1 ± 8.2	17.8 ± 22.8	11 ± 12.4	21.6 ± 33.7	15.4 ± 22.3 (1–120)
Age of first transfusion (months)	12.1 ± 18.1	20.3 ± 24.8	14.6 ± 14	22.1 ± 33.6	17.8 ± 24.1 (1–120)
Age of start of chelation (months)	38 ± 24.4	47.1 ± 40	45.3 ± 31	53.5 ± 38.4	46.1 ± 34.8 (0–168)
Height (cm)	131.3 ± 9.4	156.9 ± 11.8*	126.2 ± 9.4	150.2 ± 9*	143.5 ± 16.5 (106.2–178)
Weight (kg)	27.8 ± 5.9	44.9 ± 10.7*	24.8 ± 5	43.8 ± 9.1*	36.8 ± 12.3 (15–66)
Weight/height (%)	21 ± 3.3	28.3 ± 5.2*	19.4 ± 2.7	28.9 ± 4.7*	25 ± 5.9 (14.1–40.8)
BMI (kg/m^2)	16.2 ± 1.9	18.4 ± 2.7*	15.6 ± 1.6	19.6 ± 2.8*	17.6 ± 2.8 (13.3–26.4)
WC (cm)	61.4 ± 5.8	71.4 ± 7.4*	58.6 ± 4.8	70.8 ± 6*	66.5 ± 8.4 (49.7–87)
Triceps skinfold (mm)	9.6 ± 3.7	10.8 ± 4	10.8 ± 2.8	16.3 ± 5*	11.7 ± 4.6 (4.6–26.7)
Subscapular skinfold (mm)	6.2 ± 3.5	7.8 ± 3#	6.4 ± 2.5	13.3 ± 5.7#	8.3 ± 4.6 (3.4–23.2)
MUAC (cm)	18.1 ± 2.4	21.8 ± 2.8*	17.8 ± 1.8	23.1 ± 3*	20.5 ± 3.4 (14–30.5)
Arm muscle area (AMA)	15.1 ± 1.5	18.4 ± 2.4*	14.5 ± 1.4	18 ± 1.9*	16.8 ± 2.6 (11.6–25.6)
BMD vertebra (g/cm^2)	0.58 ± 0.08	0.75 ± 0.13*	0.53 ± 0.08	0.78 ± 0.15*	0.67 ± 0.15 (0.36–1.1)
BMC vertebra (g)	18.8 ± 4.6	35.6 ± 10.2*	16.4 ± 4	34.9 ± 10.2*	27.8 ± 12 (9.4–66.3)
Area vertebra (cm^2)	32.2 ± 4.6	46.4 ± 6.9*	30.6 ± 3.5	44 ± 6.4*	39.5 ± 9.1 (23.8–59.2)
Fracture risk vertebra	12 ± 4.4	5.8 ± 3.2#	28.1 ± 13.6	7.7 ± 6.5#	12.3 ± 11.3 (0.76–71.9)
BMD femur (g/cm^2)	0.79 ± 0.12	0.95 ± 0.13*	0.69 ± 0.09	0.84 ± 0.12*	0.84 ± 0.15 (0.52–1.2)
BMC femur (g)	12.5 ± 3.6	24.5 ± 6.8*	11.1 ± 3.2	19.4 ± 4.6*	18.1 ± 7.6 (5.5–42.4)
Area femur (cm^2)	15.5 ± 2.9	25.5 ± 4.9*	15.9 ± 3.3	22.6 ± 3.2*	20.8 ± 5.8 (7.6–39.5)
Fracture risk femur	4.1 ± 2.1	1.9 ± 1.3#	6.5 ± 3.1	2.9 ± 1.8#	3.5 ± 2.7 (0.43–14.2)

SD Standard deviation, *MUAC* mid upper arm circumference, *WC* waist circumference, *BMI* Body Mass Index, *BMD* bone mineral density, *BMC* bone mineral content

* $P < 0.05$ independent sample t test

\# $P < 0.05$ Mann–Whitney test (for boys and girls based on age group)

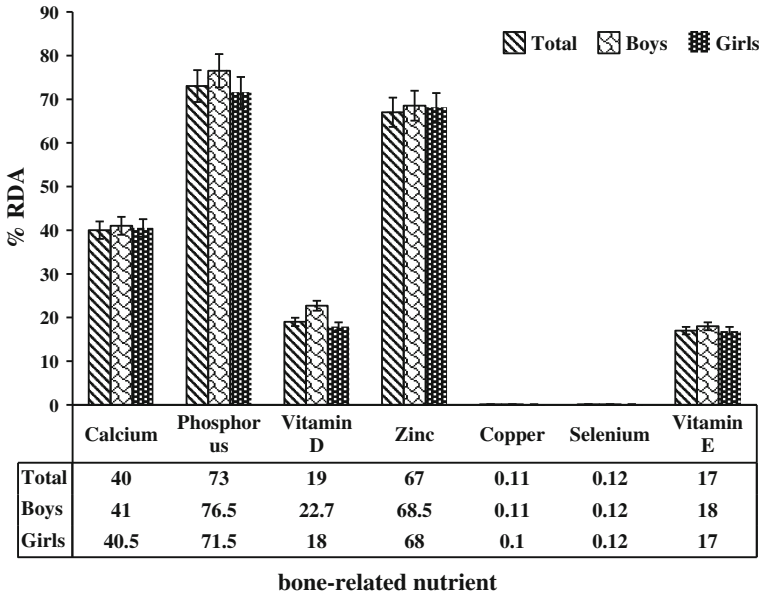

Fig. 1 Percentage of bone-related nutrient intake in subjects to RDA

Table 2 Distribution of biochemical profile of subjects according to gender and age group (presented as mean ± SD)

Characteristic	Boys ($n = 73$)		Girls ($n = 58$)		Total ($n = 131$) (range)
	8–12 years ($n = 29$)	13–18 years ($n = 44$)	8–12 years ($n = 30$)	13–18 years ($n = 28$)	
Pre-transfusion Hb (g/dl)[#]	9.4 ± 0.4	9.5 ± 0.8	9.5 ± 0.6	9.8 ± 0.6	9.5 ± 0.7 (7.8–11.4)
Serum ferritin (ng/ml)	5284 ± 6386	5232 ± 4383	4415 ± 4118	5204 ± 4565	5052.3 ± 4831.5 (200–26850)
Serum calcium (mg/dl)	9.1 ± 0.6	9.3 ± 0.7	9.1 ± 0.7	9.3 ± 0.6	9.2 ± 0.7 (7.3–11.3)
Serum phosphate (mg/dl)	4.8 ± 0.5	4.9 ± 1	4.6 ± 0.9	4.8 ± 0.7	4.8 ± 0.8 (1.2–8.7)
Serum alkaline phosphatase (U/l)	486 ± 167	392 ± 197*	501 ± 197	347 ± 197*	427.3 ± 198.8 (84–1042)
Serum TSH (μU/ml)	2.5 ± 1.4	2.4 ± 1.1	2.7 ± 1.8	2.4 ± 1.6	2.5 ± 1.4 (0.1–9.8)
T_4 (μg/dl)	8.5 ± 1.6	7.8 ± 1.7	8.3 ± 2	8.7 ± 2.2	8.2 ± 1.9 (2.8–13)
FBS (mg/dl)	89.8 ± 9.2	113.8 ± 69[#]	99.6 ± 55	108.2 ± 53	104.2 ± 54.8 (58–435)
PTH (ng/l)	18.9 ± 10.9	23.7 ± 9.3*	23.7 ± 13.7	28.2 ± 18.2	23.8 ± 13.2 (5.9–76)
25(OH)Vitamin D (nmol/l)	27.8 ± 11.2	32.8 ± 21.8	23.7 ± 16	18.4 ± 6.4	26.7 ± 16.9 (9.2–98.8)
Osteocalcin (ng/ml)	53.9 ± 16.1	46.9 ± 22.7	56.2 ± 18.8	47.05 ± 22.1	51.6 ± 19.8 (4.8–87.9)
C-telopeptide (ng/ml)	1.4 ± 0.6	1.1 ± 0.7	1.5 ± 0.4	1 ± 0.5	1.2 ± 0.6 (0.1–3.14)

TSH Thyroid stimulating hormone, *T4* thyroid hormone, *FBS* fasting blood sugar, *PTH* parathyroid hormone

* Independent Sample *t* test

[#] Mann–Whitney test (comparison between groups), significant level $P < 0.05$

Table 3 Percentage of subjects with bone-related abnormalities according to gender and age group [presented as *n* (%)]

Abnormality	Total ($n = 131$)	Boys ($n = 73$)		Girls ($n = 58$)		Normal value
		8–12 years ($n = 29$)	13–18 years ($n = 44$)	8–12 years ($n = 30$)	13–18 years ($n = 28$)	
Hypocalcaemia	29 (22)	9 (31)	6 (13.6)	9 (30)	5 (17.9)	2.22–2.70 mmol/l (≤12 years)
Normal calcium	89 (67.4)	19 (65.5)	30 (68.2)	21 (70)	18 (64.3)	2.17–2.50 mmol/l (>12 years)
Hypercalcaemia	14 (10.6)	1 (3.4)	8 (18.2)	0 (0.0)	5 (17.9)	
Hypophosphatemia	24 (18.2)	12 (41.4)	1 (2.3)	11 (36.7)	0 (0.0)	1.48–1.77 mmol/l (≤12 years)
Normal phosphate	53 (40.2)	14 (48.3)	10 (22.7)	17 (56.7)	11 (39.3)	0.90–1.45 mmol/l (>12 years)
Hyperphosphatemia	55 (41.7)	3 (10.3)	33 (75)	2 (6.7)	17 (60.7)	
Low ALP level	10 (7.7)	0	4 (9.1)	2 (6.9)	4 (14.3)	180–1200 U/l (children)
Normal ALP	110 (84.6)	28 (100)	34 (77.3)	28 (93.1)	21 (75)	100–270 U/l (>18 years & Male)
High ALP level	10 (7.7)	0 (0.0)	6 (13.6)	0 (0.0)	4 (10.7)	100–240 U/l (>18 years & Female)
Low PTH level	5 (3.4)	5 (17.2)	0 (0.0)	0 (0.0)	0 (0.0)	
Normal PTH	121 (93.2)	24 (82.8)	44 (100)	28 (93.3)	25 (89.3)	10–65 ng/l
High PTH level	5 (3.4)	0 (0.0)	0 (0.0)	2 (6.7)	3 (10.7)	
Severe VitD* deficiency	7 (5.1)	0 (0.0)	1 (2.5)	2 (6.6)	4 (10.7)	≤12.5 nmol/l
VitD deficiency	104 (79.6)	24 (82.8)	31 (70.5)	24 (80)	25 (89.3)	≤37.5 & >12.5 nmol/l
VitD insufficiency	11 (8)	4 (13.8)	5 (11.3)	2 (3.4)	0 (0.0)	37.5–50 nmol/l
VitD sufficiency	10 (7.3)	1 (3.4)	6 (13.6)	3 (10)	0 (0.0)	50–250 nmol/l

Reference Tietz textbook of clinical chemistry [31]

ALP alkaline phosphatase, *PTH* parathyroid hormone

* Misra et al. [16]

Discussion

High serum ferritin level in all of the subjects, as well as low serum hemoglobin level in the majority of them (75.8 %) reflects the inadequacy of blood transfusion and iron chelation in this study. A high prevalence of hypocalcaemia (22 %) and hyperphosphatemia (41.7 %) was observed in this study, and may reflect the incidence of hypoparathyroidism as confirmed by other researchers [18, 19]. Vitamin D deficiency (vitamin D <37.5 nmol/l) was

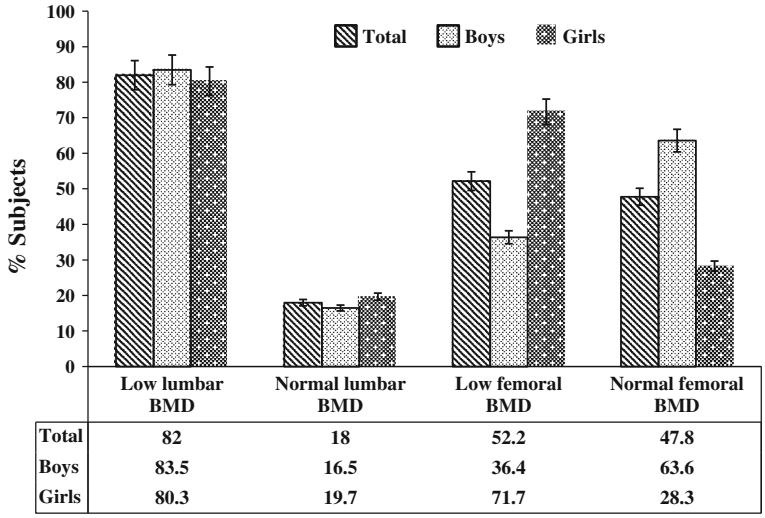

Fig. 2 Percentage of lumbar and femoral low bone mineral density in subjects (compared to Indian Database)

Table 4 Pearson correlation coefficient between bone density parameters and other parameters ($n = 140$)

Variable	BMD (lumbar)	BMC (lumbar)	BMD (femoral)	BMC (femoral)
Age (year)$^{\neq}$	0.795**	0.863**	0.604**	0.828**
Age of diagnosis (month)$^{\neq}$	0.119	0.210*	0.020	0.170*
Serum calcium (mg/dl)	0.136	0.176*	0.183*	0.218*
Alkaline phosphatase (U/l)	−0.305**	−0.266**	−0.223*	−0.253**
Serum zinc (μg/dl)	−0.136	−0.159	−0.159	−0.210*
Osteocalcin (ng/ml)	−0.426**	−0.447**	−0.360**	−0.353**
Serum C-telopeptide (ng/ml)	−0.352**	−0.377**	−0.184	−0.297**
Height (cm)	0.763**	0.878**	0.725**	0.902**
Weight (kg)	0.810**	0.909**	0.697**	0.868**
BMI (kg/m^2)	0.661**	0.691**	0.476**	0.585**
Triceps skinfold thickness (mm)	0.348**	0.323**	0.078	0.132
Subscapular skinfold thickness (mm)$^{\neq}$	0.538**	0.588**	0.297**	0.468**
Mid upper arm circumference (cm)	0.719**	0.770**	0.515**	0.660**
Arm muscle area (cm^2)	0.735**	0.817**	0.618**	0.775**
Waist circumference (cm)	0.707**	0.773**	0.582**	0.719**
Fat mass (kg)	0.605**	0.633**	0.440**	0.488**

** $P < 0.01$, * $P < 0.05$, $\neq$ Spearman Correlation

shown among 85 % of subjects, with 5.1 % having severe deficiency (vitamin D <12.5 nmol/l). This prevalence was higher than reported by other studies [20, 21]. However the differences may be related to different cut-off points used. A high prevalence of low bone mineral density in lumbar spine (82 %) and femoral area (52.2 %) of thalassemic patients in this study was in agreement with earlier studies [13, 22, 23], although the lack of control group for comparison and using the normative data of Indian children, which was the best match in terms of age, gender and phenotype, were one of the limitations of this study. Similar to previous studies, there was no significant difference between girls and boys in the prevalence of low bone density in lumbar spine. However, femoral low bone density was more common among girls (71.7 %). The other limitation of this study was the determination of pubertal status, using Tanner stage. Due to ethical prohibition, puberty was determined according to the parents' report, after instructing them with the Tanner stages characteristics.

Determination of parameters which may be associated with low bone density in high risk groups including thalassemic children and adolescents seems critical. In this study, younger age, hypocalcaemia, impaired nutritional status, especially lower weight and greater subscapular skinfold thickness were the factors associated with low bone mineral density and content in lumbar spine. Puberty

Table 5 Correlation coefficient between macro and micronutrients intake and bone density indices

Variable	BMD (l)	BMC (l)	BMD (f)	BMC (f)
Energy	0.61**	0.61**	0.57**	0.61**
Protein	0.33*	0.37**	0.32**	0.38**
Fat	0.35*	0.33*	0.23*	0.31*
CHO	0.46**	0.47**	0.52**	0.49**
Zinc	0.41**	0.37*	0.27	0.33*
Copper	0.31*	0.26	0.28	0.26
Calcium	−0.10	−0.13	−0.20	−0.10
Phosphorus	0.15	0.17	−0.001	0.17
Iron	0.41**	0.41**	0.46**	0.45*
Folate	0.17	0.15	0.09	0.08
Vit B_{12}	0.17	0.14	0.26	0.20
Vit D	−0.43**	−0.39*	−0.36*	−0.31
Vit A	0.15	0.12	0.18	0.17
Vit C	0.17	0.15	0.07	0.07
Vit E	0.34*	0.26	0.11	0.17
Selenium	0.01	0.10	0.04	0.20

BMD bone mineral density, *BMC* bone mineral content

* $P < 0.05$, ** $P < 0.01$ significant level, (l) = lumbar, (f) = femur, CHO = Carbohydrate

negatively influenced bone mineral density and content in lumbar spine. In the femoral region, younger age and shorter height were associated with low bone density and content. The factors which were associated with low femoral bone density negatively included gender and serum osteocalcin. These results were consistent with other researchers that reported age, short stature, and low serum calcium as the factors associated with low bone density among thalassemic patients. The skeleton needs calcium and phosphorus as a major structural component and mechanical loading to stimulate mineral deposition. Serum calcium and phosphorus may have a potentiating effect in bone mineral density improvement. Higher intake of zinc and iron improves nutritional status among thalassemia patients and will affect the bone mineral density and content. The higher intake of vitamin E, as an antioxidant, can suppress the harmful effect of iron overload in these patients, leading to better improvement in nutritional status and bone health [13, 24–29].

In this study, the intake of energy, macronutrients and iron was positively correlated with all anthropometric parameters as well as bone density indices. The intake of zinc and vitamin E was positively related to lumbar BMD

Table 6 Multivariate regression model predicting subject's bone health status according to bone mineral indices

Independent variables	Coefficient (B)	SE	*P* value
Model 1 ($R^2 = 0.76$, $F = 74.11$, $P < 0.001$); Outcome = BMD lumbar			
Age	0.018	0.003	<0.001
Puberty	(−) 0.154	0.031	<0.001
Model 2 ($R^2 = 0.91$, $F = 138.86$, $P < 0.001$); Outcome = BMC lumbar			
Weight	0.599	0.092	<0.001
Puberty	(−) 11.64	1.54	<0.001
Age	0.569	0.210	0.009
Subscapular skinfold thickness	(−) 0.411	0.140	0.005
Serum calcium	1.49	0.74	0.049
Model 3 ($R^2 = 0.55$, $F = 59.92$, $P < 0.001$); Outcome = BMD femoral			
Height	0.006	0.001	<0.001
Gender	(−) 0.072	0.022	0.001
Serum osteocalcin	(−) 0.001	0.001	0.020
Model 4 ($R^2 = 0.80$, $F = 143.51$, $P < 0.001$); Outcome = BMC femoral			
Height	0.295	0.048	<0.001
Age	0.394	0.188	0.040

BMD bone mineral density, *BMC* bone mineral content, *ALP* alkaline phosphatase, *SE* standard error

Model 1 adjusted for; gender, serum ALP, serum osteocalcin and C-telopeptide, height, weight, Triceps & Subscapular skinfold thickness, MUAC, AMA, WC

Model 2 adjusted for; gender, serum ALP, serum osteocalcin & C-telopeptide, height, Triceps skinfold thickness, MUAC, AMA, WC, Fat mass, age of Diagnosis

Model 3 adjusted for; puberty, age, serum osteocalcin, weight, subscapular skinfold, MUAC, AMA, WC, fat mass, serum calcium

Model 4 adjusted for; gender, puberty, serum ALP, serum osteocalcin & C-telopeptide, weight, subscapular skinfold thickness, MUAC, AMA, WC, fat mass, serum calcium, age of diagnosis, serum zinc

Puberty (1 = post-pubertal/0 = pre-pubertal), Gender (1 = boys/0 = girls)

and BMC. Bounds and his colleagues [28] confirmed in their longitudinal study that dietary factors including the intake of energy, protein, iron and zinc was positively related to healthy children's BMC. Studies on the effects of different nutrients, other than calcium, on children's bone mineral development are rare, allowing only limited comparison of results of the current study with others. Regarding the association between vitamin D intake and bone density, a meta-analyses by Winzenberg et al. [30], consists of six placebo-controlled clinical trials, showed that not only vitamin D was not beneficial for children and adolescents with normal vitamin D level, but also its effect on bone density in deficient children should be confirmed. Almost 85 % of subjects in this study had a low serum vitamin D, these results were not generalizable nor could they be compared to other studies. Several factors contributed significantly to the prediction of children and adolescent's BMD and BMC in this study. Earlier studies also found some relationships between weight, height, serum calcium and phosphorus and bone measures in children and adolescents [13, 25, 26, 28]. Studies indicating the predictors of nutritional status and bone health in thalassemic children and adolescents are rare, allowing only limited comparison with studies focusing on healthy children.

In conclusion, bone health was greatly compromised in thalassemia. Malnutrition was prevalent in thalassemic children and adolescent. More than 40 and 30 % of them suffered from short stature and underweight, respectively. Bone-related abnormalities including hypocalcaemia, hyperphosphatemia and vitamin D deficiency were very common among thalassemic patients. The prevalence of low bone mineral density might be high in vulnerable groups of children including; thalassemic children and adolescents. Low bone density could be due to the progression of disease or inadequate nutrient intake. So, bone densitometry test is suggested routinely in this vulnerable group of children. In addition, nutrition education, diet counseling and preventive supplementation therapy (calcium, zinc, vitamin D) for high risk group of children and adolescents is strongly recommended; however, this should be confirmed by interventional studies.

Acknowledgments This research project was financially supported by the Mashhad University of Medical Science Research Council and Iran National Science Foundation. The contribution of the staff of the Avicenna (Bu-Ali) Research Institute, Biochemistry and Nutrition Department of the Mashhad University of Medical Science, Iran National Science Foundation (INSF), Pharmacy faculty, Noor bone densitometry center, Sarvar clinic and lab, Sadra lab and Nutrition and Dietetics Department of the Universiti Kebangsaan Malaysia is gratefully acknowledged. Scientific editing of Prof Gordon Fern is gratefully appreciated.

Conflict of interest All authors have no conflict of interest.

References

1. Kuttikat A, Grant R, Chakravarty K (2004) Management of osteoporosis. J Indian Rheumatol Assoc 12:104–118
2. Cashman KD (2006) Milk minerals (including trace elements) and bone health. Int Dairy J 16:1389–1398
3. Bonjour JP, Ammann P, Chevalley T, Ferrari S, Rizzoli R (2003) Nutrition aspects of bone growth: an overview. In: New S, Bonjour JP (eds) Nutritional aspects of bone health. Royal Society of Chemistry, Cambridge
4. Burrows M (2007) Exercise and bone mineral accrual in children and adolescents. J Sports Sci Med 6:305–312
5. Soyka LA, Fairfield WP, Klibanski A (2000) Hormonal determinants and disorders of peak bone mass in children. J Clin Endocrinol Metab 85:3951–3963
6. Tucker KL (2003) Dietary intake and bone status with aging. Curr Pharm Des 9:2687–2704
7. Semeao EJ, Jawad AF, Stouffer NO, Zemel BS, Piccoli DA, Stallings VA (1999) Risk factors for low bone mineral density in children and young adults with Crohn's disease. J Pediatr 135:593–600
8. Henderson CJ, Specker BL, Sierra RI, Campaigne BN, Lovell DJ (2000) Total body bone mineral content in non-corticosteroid-treated postpubertal females with juvenile rheumatoid arthritis: frequency of osteopenia and contributing factors. Arthritis Rheum 43:531–540
9. Pirinccioglua AG, Akpolatb V, Koksala O, Haspolata K, Soker M (2011) Bone mineral density in children with beta-thalassemia major in Diyarbakir. Bone 49:819–823
10. Leung TF, Chu Y, Lee V, Cheng FT, Leung WK, Shing MK, Li CK (2009) Long-term effects of pamidronate in thalassemic patients with severe bone mineral density deficits. J Hemoglobin 33:361–369
11. Merchant R, Udani A, Puri V, D'cruz V, Patkar D, Karkera A (2010) Evaluation of osteopathy in thalassemia by bone mineral densitometry and biochemical indices. Indian J Pediatr 77: 987–991
12. Bekheirnia R, Shamshirsaz AA, Kamgar M, Bouzari N, Erfanzadeh G, Pourzahedgilani N, Tabatabaie SM, Shamshirsaz AA, Kimiagar M, Ezzati F (2004) Serum zinc and its relation to bone mineral density in β-thalassemic adolescents. J Biol Trace Elem Res 97:215–224
13. Shamshirsaz AA, Bekheirnia MR, Kamgar M, Pakbaz Z, Tabatabaei SM, Pourzahedgilani N, Bouzari N, Azarkeivan A, Hashemi SR, Moosavi F, Alebouyeh M, Vosough P, Kimiagar M, Shamshirsaz AA, Moradi M, Habibzadeh MR, Nobakhthaghighi N, Larijani B (2007) Bone mineral density in iranian adolescents and young adults with beta thalassemia major. J Pediatr Hematol Oncol 24:469–479
14. Terpos E, Voskaridou E (2010) Treatment options for thalassemia patients with Osteoporosis. Ann N Y Acad Sci 1202:237–243
15. International Society for the Advancement of Kinanthropometry (2001) International Standards for Anthropometric Assessment (ISAK), 1st edn. National Library of Australia
16. Misra M, Pacaud D, Petryk A, Collett-Solberg PF, Kappy M, on behalf of the Drug and Therapeutics Committee of the Lawson, Wilkins Pediatric Endocrine Society (2008) Vitamin D deficiency in children and its management: review of current knowledge and recommendations. Pediatrics 122:398–417
17. Khadilkar AV, Sanwalka NJ, Chiplonkar SA, Khadilkar VV, Mughal MZ (2011) Normative data and percentile curves for Dual Energy X-ray Absorptiometry in healthy Indian girls and boys aged 5–17 years. Bone 48:810–819
18. Najafipour F (2008) Evaluation of endocrine disorders in patients with thalassemia major. Int J Endocrinol Metab 2:104–113

19. Salama OS, Al-Tonbary YA, Shahin RA, Eldeen OA (2006) Unbalanced bone turnover in children with beta-thalassemia. J Hematol 11:197–202
20. Wood JC, Claster S, Carson S, Menteer JD, Hofstra T, Khanna R, Coates T (2008) Vitamin D deficiency, cardiac Iron and cardiac function in thalassaemia major. Br J Haematol 141:891–894
21. Fung EB, Aguilar C, Micaily I, Haines D, Lal A (2011) Treatment of vitamin D deficiency in transfusion-dependent thalassemia. Am J Hematol 86:871–873
22. Yazigi A, Maalouf G, Inati-Khoriati A, Tamim H, Saab C (2002) Bone mineral density in beta—thalassemic Lebanese children. J Musculoskelet Neuron Interact 2:463–468
23. Eren E, Yilmaz N (2005) Biochemical markers of bone turnover and bone mineral density in patients with beta-thalassaemia major. Int J Clin Pract 59:46–51
24. Rashidi MR, Aboomardani M, Rafraf M, Arefhosseini SR, Keshtkar N, Joshanghani H (2011) Effects of Vitamin E and zinc supplementation on antioxidants in beta thalassemia major patients. Iranian J Pediatr 21:8–14
25. Kardinaal AFM, Hoorneman G, Vananen K, Charles P, Ando S, Maggiolini M, Charzewska J, Rotily M, Deloraine A, Heikkinen J, Juvin R, Schaafsma G (2000) Determinants of bone mass and bone geometry in adolescent and young adult women. Calcif Tissue Int 66:81–89
26. Palasuwan A, Soogarun S, Wiwanitkit V, Luechapudiporn R, Pradniwat P, Lertlum T (2006) Preliminary study of the effect of vitamin e supplementation on the antioxidant status of hemoglobin-e carriers. Southeast Asian J Trop Med Public Health 37:184–189
27. Malina RM, Bouchard C (2004) Growth, maturation, and physical activity. Human Kinetics, Champaign
28. Bounds W, Skinner J, Carruth BR, Ziegler P (2005) The relationship of dietary and lifestyle factors to Bone Mineral Indexes in children. J Am Diet Assoc 105:735–741
29. Tantawy AA, El Kholy M, Moustafa T, Elsedfy H (2008) Bone mineral density and calcium metabolism in adolescents with Beta-Thalassemia Major. Pediatr Endocrinol Rev 6:132–135
30. Winzenberg T, Powell S, Shaw KA, Jones G (2011) Effects of Vitamin D supplementation on bone density in healthy children: systematic review and meta-analysis. Br Med J 342:c7254
31. Burtis CA, Ashwood ER, Teitz NW (1999) Teitz Textbook of Clinical Chemistry, 3rd edn. Saunders, Philadelphia