

Bone mineral density in beta - thalassemic Lebanese children

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Abstract

Osteoporosis has been described extensively in adult thalasseemics. Fewer studies have been reported in thalassemic children. In this article, we measured the bone mineral density (BMD) of Lebanese beta-thalassemic children before institution of a balanced transfusion-chelation regimen, in comparison with that of healthy controls, and studied its correlation with various demographic and biological parameters. Both groups, controls and thalasseemics were comparable with respect to age, sex, socioeconomic and regional distribution. On the other hand, thalasseemics had a significantly lower height age ($p < 0.001$), lower bone age ($p = 0.001$), lower sexual maturation ($p = 0.004$), lower absolute BMD values and larger negative BMD-Z scores ($p < 0.001$). Within the thalassemic group, BMD correlated significantly with luteinizing hormone (LH) and follicle stimulating hormone (FSH), estradiol and testosterone values, as well as with the pretransfusion hematocrit, but not with other endocrine or bone metabolism parameters. We conclude that Lebanese beta-thalassemic children have a significantly lower BMD than their healthy counterparts due, in part, to their slower physical development. A major contributor seems to be the low-transfusion regimen followed by these patients, as well as the endocrine dysfunction which was detected in about 25% of them.

Keywords: Bone Mineral Density, Children, Osteoporosis, Thalassaemia

Introduction

Osteoporosis has been extensively described to occur in beta-thalassaemia^{1,2}, especially in adult survivors. However, the role of various factors in its causation is not clearly identified. Some studies incriminate hypogonadism³, while others stress the effect of bone marrow hyperactivity⁴.

Since our thalassemic children are living longer, it was important for us to evaluate their bone status, in order to offer them an optimal treatment, at an age when bone mineral density constitutes their capital for a healthy osseous development in the future. Therefore, in this study, we compared a group of beta-thalassemic children at the time of their entry to the Chronic Care Center for management, with matching healthy controls and correlated their BMD with various demographic and biochemical parameters.

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Subjects and methods

Fifty-two healthy children, 9 to 18 years of age, were randomly selected from 15 schools in various regions of the country, to serve as controls : 6-7 children were chosen by lottery from each of the eight school grades that cover the 9 to 18 years age range. Two were excluded: one because of a history of multiple fractures, and one because of repeated prolonged courses of corticosteroid therapy for severe asthma. Parents were requested to complete a questionnaire as to their place of origin/residence and nutritional habits (mainly intake of proteins and dairy products). All the subjects underwent a physical examination performed by two pediatricians who recorded their height, weight and sexual maturation score (Tanner staging). A bone age and spinal bone mineral density (BMD) measurements were done on all.

Twenty-nine homozygous beta-thalassemic children, seen for the first time at the Chronic Care Center, also 9 to 18 years old, were randomly assigned by order of admission to undergo the same procedures as the controls, in addition to a set of biologic tests: an endocrine profile (serum thyroid

	Controls n=50	Thalasseemics n=29	<i>p</i> Value
Height (cm)			
males	156.9 ± 15.4	139.7 ± 10.1	< 0.001
females	154.4 ± 9.8	136.9 ± 12.6	< 0.001
Weight (Kg)			
males	50.2 ± 16.6	34.8 ± 6.8	0.001
females	48.4 ± 12.7	34.8 ± 8.9	0.002
Height age(years)	13.8 ± 2.6	10.6 ± 1.9	< 0.000
Bone age (years)			
Sempé	13.1 ± 3.1	10.9 ± 1.9	0.001
Greulich & Pyle	13.1 ± 3.0	10.9 ± 1.9	0.001
Sexual maturation			
Prepubertal	13	17	0.004
BMD g/cm²			
mean ± SD	0.873 ± 0.211	0.626 ± 0.076	0.001
BMD Z-Score			
Z-CA*	- 0.96	-3.50 ± 1.51	< 0.001
Z-BA**	-1.04	-1.98 ± 0.94	0.001
Z-HA***	-1.27	-1.79 ± 0.73	0.04
* BMD Z-CA = BMD Z-Score when BMD is plotted against the chronological age. ** BMD Z-BA = Same when plotted vs. the bone age. *** BMD Z-HA = Same when plotted vs. the height age.			

Table 1. Demographic and BMD values of controls and thalasseemics.

stimulating hormone [TSH], free thyroxin [FT4], follicle stimulating hormone [FSH], luteinizing hormone [LH], insulin growth factor [IGF1], testosterone for boys, estradiol for girls), a hematologic evaluation (pre-transfusion hematocrit, serum ferritin), and measurement of parameters of bone metabolism (serum calcium, phosphorus, alkaline phosphatase, osteocalcin, parathyroid hormone [PTH]). FSH and LH levels were analyzed by the two-site immunoradiometric assay (IRMA-Mat®) using the Byk-Sangtec Diagnostica GmbH & Co. KG; testosterone levels were measured by RIA-mat® Testosteron c.t. from Byk-Sangtec Diagnostica GmbH & Co. KG; estradiol was measured by the enzyme-linked fluorescent assay (ELFA) technique using Vidas®-Bio Merieux s.a. IGF1, FT4, TSH and PTH were analysed by radioimmunoassay (RIA).

The height age was defined by plotting the height on a standard growth curve⁵. The bone maturation and calculated average bone age (BA) on the X-ray of the left hand and

wrist was read and double-checked by both the Sempé⁶ and Greulich & Pyle methods⁷, by the endocrinologist who was blind as to the age, Tanner stage and category of the subject (control or thalassemic). The mean value between the two readings was used for further calculations, since the 2 methods correlated perfectly with each other. FSH, LH, testosterone and estradiol were categorized as normal or high/low for their respective bone age. TSH, FT4 and bone metabolism parameters were categorized as normal or high/low for their respective chronological age.

The BMD at the L1-L4 region was determined by the DEXA machine (DPXL-LUNAR) and recorded as the Z-score relative to the chronological age (BMD Z-CA), the bone age (BMD Z-BA) and the height age (BMD Z-HA), in reference to a standard population⁸.

Data was entered and analyzed on a PC, using the SPSS for Windows software. Frequency distributions were used to present the characteristics of both populations. With cate-

Parameters	number/ determined	BMD Z-CA	<i>p</i> Value
IGF1	Normal	6/16	-3.69
	Low	10/16	-3.32
TSH	Normal	26/28	-3.47
	High	2/28	-4.42
FT4	Normal	27/28	-3.47
	Low	1/28	-4.26
LH	Normal	21/27	-3.24
	Low	6/27	-4.82
FSH	Normal	16/22	-3.24
	Low	6/22	-4.82
Testosterone	Normal	14/17	-2.9
	Low	3/17	-5.3
Estradiol	Normal	8/11	-3.7
	Low	3/11	-4.4

Table 2. Frequency of abnormal hormone values in thalassemics and their relation to BMD.

gorical data, cross-tabulations were made to detect the differences between the two populations. The significance level was determined by the Pearson Chi-Square values, and the level was set at 5%. For continuous variables, the test statistic used was the student's t-test to detect any significant differences between thalassemics and the comparison group.

Multiple regression analysis was applied to detect relation between selected variables and BMD. The same significance level was kept.

All parents signed an informed consent form. The project was approved by the Ethical Committee of both the Saint-George Hospital and the Chronic Care Center.

Results

The 50 healthy (25 boys and 25 girls) and 29 thalassemic subjects (17 boys and 12 girls) had a mean age of 13.4 ± 2.6 years and 13.6 ± 2.7 years respectively ($p=NS$).

Table 1 shows that thalassemic boys and girls were shorter and weighed less than their healthy counterparts; they had a significantly lower height age, lower bone age by either Sempé or Greulich and Pyle methods, lower rate of sexual maturation, lower mean BMD values; their BMD showed a larger negative deviation from the mean when plotted against their chronological age (BMD Z-CA), and a still significant although narrower deviation when plotted against their bone age (BMD Z-BA) or their height age (BMD Z-HA). The mean BMD Z-CA of thalassemic children was -3.50 ± 1.51 , BMD Z-BA was -1.98 ± 0.94 and BMD Z-HA was -1.79 ± 0.73 . The difference was statistically significant between BMD Z-CA and BMD Z-HA ($p < 0.0001$), but not between BMD Z-BA and BMD Z-HA ($p = 0.42$).

Table 2 shows the frequency of abnormal hormonal values in thalassemic children and how they relate to their BMD. Thyroid dysfunction was very rare, only 2 subjects having high TSH, and one a low FT4. There was no correlation between IGF1 and BMD. On the other hand, 6 out of 22 thalassemics

Variable	Beta coefficient (SE)	p value
Constant	-3.12 (1.4)	0.05
IGF1	0,96(0.67)	NS
LH*	-2.02 (0.81)	0.03
TSH	0.50 (1.27)	NS

*R² = 0.382

Table 3. Correlation of hormone values with BMD by multiple regression analysis.

had low FSH, 6 of 27 had low LH, 3 of 17 boys had low testosterone and 3 of 11 girls had low estradiol for their respective bone age. Low FSH, low LH and low testosterone correlated significantly with low BMD. Girls with low estradiol values also had lower BMD than those with normal estradiol, but the difference was not statistically significant, possibly because of the small number of subjects. LH, FSH, testosterone and estradiol were perfectly correlated with each other (Pearson correlation coefficient = 1); so LH was selected for multiple regression analysis because it was the variable with the lesser number of missing values. In this model, FT4 was not considered, because only one subject had a low FT4 level. The results show that LH (and therefore FSH, testosterone and estradiol) correlated significantly with BMD Z-CA (Table 3).

Twenty-two out of 27 thalassemics had elevated osteocalcin blood levels indicating high osseous turnover, with values ranging from 50 to 126.5 ng/ml. The only significant correlation was a negative one between elevated serum phosphorus and low BMD ($p=0.01$). Serum phosphorus values were high in 11 of 27 patients, 3 out of 26 had low calcium levels, 18 out of 27 had high alkaline phosphatase, and only one out of 27 had a low PTH value, the others being normal. 1,25-dihydroxy Vitamin D (1,25 [OH]₂ D) was not determined. The same results were obtained by multiple regression analysis (Table 4).

The hematocrit values at which our thalassemic children were transfused ranged from 24.5% to 30%, with a mean value of $28.37\% \pm 1.65\%$. We found that children transfused at a higher hematocrit level had a significantly higher BMD, the correlation coefficient being equal to 0.42 with a p value of 0.02. In order to assess the adequacy of chelation of our thalassemic children, we determined serum ferritin in 28 of them. In none was the ferritin value below 1000 ng/ml. Four ranged between 1000 ng/ml and 2000 ng/ml, six between 2000 and 4000 ng/ml, nine between 4000 and 7000 ng/ml and nine above 7000 ng/ml.

There was no significant correlation between BMD and ferritin levels ($p=0.1$), nor between ferritin values and sexual hormone levels ($p=0.7$).

Discussion

The histological features of bone in Thalassemia Major are complex and the roles of various factors in their pathogenesis are not clearly identified. Besides the classical skeletal changes⁴, osteoarthropathy² and osteoporosis^{1,9,10} have been described extensively, especially in adults. Fredericks et al.¹¹ assessed trabecular bone mineral concentration in the vertebrae of 132 children including 54 thalassemics by quantitative computed tomography. Rioja et al.¹² studied iliac crest biopsies from 17 thalassemic children with severe skeletal changes; they detected histochemically iron deposits at various sites and mostly severe cortical bone changes including fissures and focal mineralization defects. BMD has been measured and found decreased by different authors in adult thalassemics^{3,13}. In children, Soliman et al.¹⁴ demonstrated a correlation between IGF1 and BMD, and Filosa et al.¹⁵ emphasized the role of disease severity on bone in thalassemics.

Our results show that Lebanese thalassemic children have delayed growth and sexual maturation compared to matched controls, a finding in accordance with another study¹⁶ where about one third of the subjects were below the 3rd percentile for height, 74.5% of children older than twelve years were still prepubertal, and almost all had delayed bone age. This delay in growth and puberty, by diminishing muscle development and subsequently bone stimulation, accounts for the observed differences between controls and thalassemics that are much wider in BMD Z-CA than in BMD Z-BA and BMD Z-HA. Thalassemia seems to affect BMD through its effects on growth and development of the patients as well as through a direct role in skeletal metabolism. This is further shown by the significant difference between BMD Z-CA and BMD Z-HA of thalassemic children.

Multiple hormonal deficiencies have been described in thalassemic children, as a result of hemochromatosis, the most common being abnormalities of the hypothalamic-pituitary-gonadal axis, resulting in decreased gonadotropic function and depressed growth hormone values, as well as evi-

Variable	Beta coefficient (SE)	p value
Constant	-4.37 (2.76)	NS
Osteocalcin	0.41 (0.74)	NS
Serum Calcium	-0.83 (1.15)	NS
Serum Phosphorus*	-2.31 (0.73)	0.005
Alkaline Phosphatase	0.058 (0.59)	NS
PTH	2.08 (1.7)	NS

* $R^2 = 0.448$

Table 4. Correlation of bone metabolism parameters with BMD by multiple regression analysis.

dence of primary hypothyroidism¹⁷. Our data show a rare occurrence of thyroid dysfunction, but a significant number of children with probable hypogonadotropic hypogonadism; also IGF1 (somatomedin C) was low in 10 of the 16 subjects in whom it was determined, reflecting poor growth and nutritional status; no stimulation tests were performed, since we did not have at this point sufficient data concerning the growth velocity of these patients. Furthermore, our findings corroborated those of Anapliotou et al.³, Bisbocci et al.¹³ and Morabito [unpublished data] in adults, namely the significant correlation between gonadal hypofunction and low BMD.

The elevated osteocalcin level in the majority of our subjects is a reflection of their high osseous turnover as well as the fact that the majority were prepubertal, hence in a stage of bone mass accumulation. However no correlation with BMD was found.

Adult thalassemics have been found to have significantly low serum PTH, 25(OH)D₃ and 1,25-(OH)₂D₃¹⁴. In our study, only one out of 27 had a low serum PTH, but the elevated level of serum phosphorus in 11 patients, that correlated significantly with low BMD, could be an early index of hypoparathyroidism antedating the low PTH values, and contribute to the osteopenia¹⁸.

The mean pre-transfusion hematocrit value of $28.37 \pm 1.65\%$ in our thalassemics places them in the low transfusion regimen category according to Modell et al.¹⁹, a factor that seems to have a bearing on their bone rarefaction, since it was found that the lower the pre-transfusion hematocrit, the lower the BMD. This finding is in agreement with the theory that one of the mechanisms of osteoporosis in thalassemics is the bone marrow expansion⁴ secondary to the anemia. This is further substantiated by the study of Scutellari et al.²⁰ who demonstrated that a high transfusion

regimen begun early in the life of thalassemics will prevent the development of abnormal rib changes including osteoporosis, and by Katz et al.⁹ who found that keeping the hemoglobin level above 9.0 g/dl reduced osteoporosis and the incidence of fractures. It is also known that osteoclasts are derived from specialized bone cells in the bone marrow²¹. It may be postulated that the continuous regeneration of the bone marrow induces increased osteoclastic activity and secondary bone destruction.

It is relevant to note that the study was conducted early after the creation of the Chronic Care Center where these patients are enrolled, and hence before an adequate transfusion chelation program was implemented. This explains, at least in part, the poor chelation results shown in our study, where none of the subjects had ferritin values less than 1000 ng/ml, while nine had values in the dangerous zone (i.e. > 7000 ng/ml), as classified by Modell¹⁹. However, this poor chelation, and therefore high iron overload, did not correlate significantly with low BMD.

We did not find either any statistically significant correlation between ferritin values and the levels of sex hormones.

Conclusion

Our results show that Lebanese beta-thalassemic children are shorter and less sexually mature than their healthy counterparts, and have a lower BMD compared to matched controls. The delay in growth and development seems to play a major role in the observed low BMD, a fact that allows us to recommend that BMD in thalassemics be assessed in relation to their bone age or height age rather than their chronological age. The low transfusion regimen and the hypogonadal function which was encountered in about a quarter of the subjects were found to be associated with a lower BMD.

Adoption of a high transfusion regimen will hopefully limit the bone marrow hyperactivity and expansion in addition to achieving better growth and development. But it should be coupled with a better chelation program, since hemochromatosis can be a cause of endocrine dysfunction in thalassemia, a fact, however, not corroborated in our study. Reassessing the BMD at a future date in relation to the same parameters, after implementation of a better transfusion-chelation-hormone replacement program, might be useful in the evaluation of our management protocol.

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