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Original Research Article

Biochemical Markers of Bone Disease in Patients with β-Thalassemia Major in the Center of Hemoglobinopathy Lushnja, Albania

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ABSTRACT

Aim of the study: To evaluate osteopathy in Albania thalassemia patients by bone mineral densitometry (BMD) and markers of bone.
Methods: We studied 47 thalassemic patients (25 male 22 female). We evaluated height, weight, Hb, ferritin, PTH, Vitamin D, CTX, N-MID-Osteocalcin, Ca, P, BMD.
Results: Of 47 patients, 3 had osteoporosis,8 had osteopenia by z-score of DEXA. 62% had elevated B-Cross Laps, 28% had high osteocalcin, 50% had low vitamin D. We found strong correlation between serum PTH and CTX (r=0.676,p=0.000); PTH and osteocalcin (r=0.815,p=0.000); and CTX and osteocalcin (r=0.695,p=0.000).
Conclusion: We conclude that BMD should do annually and biomarker of bone turnover is helpful for early diagnosis to prevent morbidity.

Keywords: DEXA, Vitamin D, Osteoporosis, Thalassemia, B-CrossLaps

INTRODUCTION

Thalassemia major (TM) is a hereditary hemoglobinopathy causing increased eritropoiesis and expansion of the bone marrow cavity. As a consequence, there is a reduction in trabecular bone tissue resulting in osteopenia/osteoporosis. [1] These patients are prone to fractures. Osteoporosis is a universal medical problem, affecting both genders. It is generally accepted that its main causes are: (1) aging, (2) genetic disorders of osteogenesis, (3) lack of certain nutritional elements or physical activity,(4) and endocrine disorders mainly estrogen deficiency. [2]

Patients with thalassemia present skeletal bone disorders due to a medullary expansion and cortical thinning. Treatment with chelating agents and transfusion programmes, have significantly prolonged survival of TM. Quantification of bone disease in TM is best performed using Ultrasound densitometry and the determination of various markers of bone formation and resorption. [3-5]

MATERIALS AND METHODS

Subjects

A total of 47 patients affecting by thalassemia major admitted to the Center of

Hemoglobinopathy Lushnje, Albania were enrolled in this study, 25 male and 22 female, median ages 20.9 (range 12.7-55years).

Informed written consent has been obtained from all participants enrolled in this study. All patients had started blood transfusion therapy since early childhood and only 41 patients had been undergoing periodical transfusions. Moreover, 41 patients were subjected to an iron chelation program with deferoxamine (4 pts), deferasirox (29pts) and deferiprone (8pts).
BMD was measured in all patients by a ultrasound densitometer Sonost 3000.

Bone density values were expressed as T-score. T-score was calculated as standard deviation score (SDS) from normal reference population database. Date were classified according to WHO 1994 report: osteoporosis was defined as T-score below -2.5 SD, osteopenia as T-score between -1 and -2.5 SD, T-score >-1= normal. [6,7]

Biochemical assays

Fasting blood samples were taken for the measurement of biochemical panel and marker of bone turnover. Calcium (Ca, normal range 8.2-10.4 mdl); Phosfor (P, normal range 2,5-4,5 ng/ml); parathyroid hormone (PTH ;15-65 pg/ml); Vitamin D total, normal range>30ng/ml; N-MID-Osteocalcin (OC; normal range 11-43 ng/ml); Ferritin, normal range 15-100 ng/ml; Betta Cross Laps ,normal range<0.3 ng/ml. Blood cell count was performed by Mythic 18. Biochemical panel were determined by autoanalyser A-15, bone markers were measured by ECLIA (electrochemiluminescene's immunoassay) method using Elexys 2010 by Roche Diagnostic. We measured CTX as a marker of bone resorption and serum vitamin D total and N-Mid-Osteocalcin as bone formation markers.

Statistical analysis

Multivariable Cox proportional hazards models were used where applicable. Statistical evaluation was performed using JMP 10 of SAS software (SAS Institute, Cary, NC, USA). Associations of categorical baseline variables were tested using x2 and Fisher's exact tests. Comparisons of continuous baseline, outcome, and treatment-related data were tested using Wilcoxon rank-sum test. $p<0.05$ was taken as significant.

RESULTS

Anthropometric characteristics and biochemical parameters measured from serum of patients are recorded in Table 1.

Table1. Mean, median, SD and range of all parameters studied

Parameters	Normal Range	Minimum	Maximum	Mean	Std. Deviation	Median
Hb (g/dl)	11-17g/dl	5.40	9.00	7.2319	.76588	
WEIGHT(kg)		30	80	51.17	11.687	51.17
HEIGHT(cm)		140	178	160.55	9.720	160.5
T-score	-1 +1	-3.3	1.8	-.253	1.1296	-0.131
CTX (ng/ml)	<0.3 ng/ml	.146	2.410	.70443	.418304	0.615
PTH (pg/ml)	15-65 pg/ml	8	1051	85.21	161.068	50.25
TSH(uiu/ml)	0.274.2uiu/ml	.057	8.500	2.86440	1.619826	2.7
FT4(pmol/l)	12-22 pmol/l	1.0	24.3	17.228	3.6804	17.7
Ferritin(ng/ml)	10-110 ng/ml	100	4900	1189.45	1058.898	910
Vitamin D(ng/ml)	>30 ng/ml	6.86	70.00	28.9238	10.85691	29.7
Osteocalcin(ng/ml)	11-43 ng/ml	6.89	300.00	39.7540	44.73147	27.3
Age (years)		12.7	55	23.67	10.3	20.32
Calcium (mg/dl)	8.5-10 mg/dl	6.6	8.8	7.9	5.3	8,2
Phosphorus(mg/dl)	2.5-4.5 mg/dl	2.1	4.7	3.7	2.2	3.85

Of 47 patients, only three had osteoporosis (6.4%) and 8 (17%) had osteopenia by T-score of Ultrasound densitometer Sonost. PTH levels were higher in 12 patients (25%) . These patients had low T-Scores, low vitamin D and high N-MID-Osteocalcin. 62% of patients had BcrossLaps >0.3 ng/ml. 28% had high value of N-MID-Osteocalcin and 50% had low level of vitamin D. We found a correlation between CTX and N-MID-Osteocalin (r=0.68). Only one patient had very high PTH (1051 pg/ml) with N-MID-Osteocalcin 300 ng/ml and T-scores -3.3 SD, vitamin D 13.82 ng/ml , CTX 2.4 ng/ml.

We divided 47 patients in 2 groups: (group-1 normal DEXA n=35 pts) and group -2 abnormal DEXA n=12 and we presented the statistical analysis of biochemical indices between the two groups in table 2. We noted that there was a statistically significant in PTH between two groups. ($p<0.05$).

Table 2: Statistical analysis of studied parameters in two groups:

	Reference range	Normal BMD (n=35) median	Abnormal BMD (n=12)median	P value	significance
Calcium	8.5-10.5 mg/dl	8.85	8.7	0.413	No
Phosfor	2,5-4,5 mg/dl	3.85	3.8	0.674	No
PTH	15-65 ng/ml	45.56	87.15	0.01	YES
Vit D	>30 ng/ml	29.57	31.9	0.55	No
Osteocalcin	11-43 ng/ml	27.3	26.3	1	No
CTX	>0.3 ng/ml	0.616	0.558	0.81	No
Ferritin	10-110 ng/ml	880	1000	0.9	No

Bivariate Pearson correlation analyses between age and bone markers parameters are summarized in table 3.

Table.3: Correlations

		AGE	PTH	CTX	Vitamin D	Osteocalcin
AGE	Pearson Correlation	1	.430**	-.079	.132	.224
	Sig. (2-tailed)		.003	.596	.378	.129
	N	47	47	47	47	47
PTH	Pearson Correlation	.430**	1	.676**	-.237	.815**
	Sig. (2-tailed)	.003		.000	.108	.000
	N	47	47	47	47	47
CTX	Pearson Correlation	-.079	.676**	1	-.178	.695**
	Sig. (2-tailed)	.596	.000		.232	.000
	N	47	47	47	47	47
Vitamin D	Pearson Correlation	.132	-.237	-.178	1	-.201
	Sig. (2-tailed)	.378	.108	.232		.175
	N	47	47	47	47	47
Osteocalcin	Pearson Correlation	.224	.815**	.695**	-.201	1
	Sig. (2-tailed)	.129	.000	.000	.175	
	N	47	47	47	47	47

**. Correlation is significant at the 0.01 level (2-tailed).

DISCUSSION

There are several factors involved in the development of osteoporosis in thalassemia patients. Among them is chronic hypoxia, medullar expansion, vitamin D deficiency and iron accumulation. [8]

Low pretransfusion hemoglobin concentrations demonstrate that the patients included in this study suffered from chromic hypoxia. (Hb 7.1 gr/dl)

In the study of Rashid Mershant, [9] 62 % had low vitamin D suggesting either nutritional deficiency or defective

hydroxylation of vitamin D in liver due to hemochromatosis as all patients had high ferritin levels, while in our study 50% of patients had low level of vitamin D, but not very high level of ferritin.

In the present study, 25% had hyperparathyroidism, which could be explained by low level of vitamin D (50%).

Karimi at al [10] studied BMD by DEXA in Iranian patients and found significant correlation between low bone mass and low Hb level, we didn't. In our study (and in the Karimi's study) BMD was not influenced by gender, chelation therapy and biochemical parameters.

Yazigi et al [11] had 3,7% patients with hypoparathyroidism, but elevated phosphorus value that correlated with low BMD. In our study we had 4% of patients with hypoparathyroidism and normal value of phosphorus.

In our study, 62% of patients had high level of β-CrossLaps. These findings support the hypothesis that osteoporosis in thalassemia results from bone resorption.

In our study, only one patient had value out of range (very high PTH,N-MID-osteocalcin and low vitamin D), probably due to renal failure.

There was a statistical significance (p=0.01) between chronological age and BMD scores in the present study and a negative correlation (r-0.5) between them; while the Studies by Benigno et a 2003l [12] showed relationship between BMD Z scores and chronological age but, without statistical significance.

In our study, BMD values show significant differences between men and women; this finding was in contrast with the reports of Voskaridou et al. and Giardina and Grady, [4,13-15] but it was in agreement with the findings of Jensen et al [17] who reported that the bone lesions in thalassemia are more frequent and more prominent in males.

In the study of Pirinccioglu et al [16] CTX was positively correlated with octeocalcin but, it was not significant (r=0.292, p=0.069) in our study, they were positively correlated and significantly(r=0.695,p=0.000). In our study, we found strong correlation between serum PTH and CTX (r=0.676,p=0.000); PTH and osteocalcin (r=0.815,p=0.000); and CTX and osteocalcin (r=0.695,p=0.000)

CONCLUSIONS

Patients with thalassemia should receive optimal transfusion to prevent excessive bone expansion. Undernourishment, hypovitaminosis D and secondary hyperparathyroidism may lead to osteopathy even at an early age BMD should be done annually and the measurement of biochemical markers is important to prevent osteoporosis.

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