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Dual X Ray absorptiometry is superior in detecting bone mineral density alterations in adult patients with thalassaemia major compared to quantitative computed tomography

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ABSTRACT

Purpose: Patients with beta thalassaemia major are prone to develop osteoporosis during prolonged lifespan despite effective and regular chelation treatment. The present study aims to assess the bone mineral density in thalassaemic patients with dual x ray absorptiometry (DXA) and quantitative computed tomography (QCT), to assess the diagnostic performance of each method and correlate bone alterations with various clinical parameters and bone markers.

Material and Methods: The study included 75 homozygous thalassaemic adult patients (31 men, 44 women, age range 22-49 years) that were in regular transfusion and chelation therapy. All patients underwent DXA and QCT measurements and laboratory examinations.

Results: First, we examined the relationship of lumbar Z scores at the lumbar spine between DXA and QCT measurements and there was no evidence of agreement between the two densitometric methods, in almost all cases. DXA measurements of the lumbar spine and femoral neck detected 24/75 patients (32%) and 10/75 patients (10%), respectively, below the expected range for age, indicative of diminished bone mineral density. Regarding bone markers and BMD, we found that ferritin has a statistically significant positive correlation with BMD of the femoral neck in both males and females. Similarly, osteocalcin has a statistically significant positive correlation with BMD of the femoral neck in male patients only.

Conclusions: Osteoporosis should be assessed only with DXA and should be taken into clinical consideration for monitoring and therapy in adult patients with thalassaemia.

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KEY WORDS
bone mineral density; dual x ray absorptiometry; quantitative computed tomography; thalassaemia major

Introduction

Beta thalassaemia major is a genetic disorder characterized by decreased or absent production of b-globin chains leading to abnormal haemoglobin formation, chronic haemolytic anaemia, ineffective erythropoiesis, compensatory haematopoietic expansion, hypercoagulability, metabolic bone changes and increased intestinal iron absorption [1,2]. Conventional management primarily relies on a regular hyper-transfusion regime and iron-chelation therapy. Osteoporosis remains one of the serious complications, even in patients fully complied with red blood cell (RBC) transfusions and chelation treatment [1,3].

DXA is the standard of reference regarding the estimation of bone mineral density (BMD). Alternative methods, such as quantitative computed tomography (QCT) and peripheral quantitative computed tomography (pQCT) can be used for assessment of BMD as well as bone strength [4,5]. Based on previous publications having studied various osteoporotic populations, it has been shown that BMD values measured by QCT are usually higher than those measured by DXA [6,7]. On the contrary, some others have found no statistically significant difference between the two methods. This observation may be reversed among patients with decreased BMD with systemic thalassaemia. It has been suggested that the increased iron accumulation in the over-expended red bone marrow in thalassaemic patients, may influence BMD measurements of the vertebral bodies due to "spilling over" effect of the iron in the bone marrow. This observation is particularly important for patients with thalassaemia major in whom an abnormal bone turnover has been well documented [8].

Bone demineralization in thalassaemia is the endpoint of a complex path of defects that coexist during skeletal maturation and adulthood. Several factors seem to contribute to low BMD, including genetic susceptibility, partial persistence of ineffective erythropoiesis, various endocrine dysregulations, negative effects of iron overload and/or chelation therapy on osteoblast function [1,2,6,9].

In the present study we aimed to explore the diagnostic capabilities of QCT and DXA measurements in lumbar spine and hip in an adult group of patients with b-thalassaemia major and correlate various clinical parameters as well as bone markers with our findings, with emphasis on the clinical relevance of possible increased fracture risk.

Table 1. Laboratory measurements

Parameter	Mean ± SD
Ferritin (ng/mL)	1295.2 ± 1630.7
Calcitonin (ng/mL)	2.19 ± 0.95
Osteocalcin (ng/mL)	8.9 ± 4.4
PTH (pg/mL)	36.6 ± 19.3
IGF-1 (ng/mL)	126.9 ± 88.5
Vitamin D	18.2 ± 7.76

Material and Methods

A. Patients

This is an observational, cross-sectional study. Patient recruitment was performed by the referral clinicians. Our study group was formed by a total of 75 patients (31 men, 44 women, median age 35 years old, age range 22-49 years). Mean height of the patients was 165 cm, mean weight was 64 kg and mean Body Mass Index (BMI) was 22.7. All patients were diagnosed with beta-thalassaemia major and were on stable transfusion regime. Splenectomy has been performed in 22/75 (45.3%) patients. Another subgroup of 13 patients (13/75, 17.3%) had a history of bone fracture, either in upper or lower limb.

Blood samples from all patients in the above study group were collected for the measurement of serum

Table 2. Correlation Tests between BMD and parameters of bone turnover in patients with thalassaemia

Gender	DXA						
	Neck Z Score	FERRITIN	CALCITONIN	OSTEOCALCIN	PTH	IGF-1	VITD
Male Female		0.39 (0.03)[Ŧ] 0.22 (0.15)	0.13 (0.45) -0.14 (0.35)	-0.41 (0.02)[Ŧ] 0.04 (0.78)	0.18 (0.32) 0.09 (0.54)	-0.03 (0.867) 0.002 (0.98)	-0.11 (0.55) 0.12 (0.44)
	L1-L4 Z Score	FERRITIN	CALCITONIN	OSTEOCALCIN	PTH	IGF-1	VITD
Male Female		0.23 (0.21) 0.37 (0.01) [Ŧ]	0.24 (0.19) -0.02 (0.91)	-0.26 (0.16) -0.06 (0.69)	0.01 (0.61) 0.234 (0.18)	-0.24 (0.19) -0.11 (0.49)	0.09 (0.64) -.024 (0.12)

[Ŧ] *Statistically Significant, p<0.05*

Values between -0.3 and -0.7 indicate a moderate negative linear relationship through a fuzzy-firm linear rule.

biochemical parameters including ferritin and various markers of bone turnover. Calcitonin, osteocalcin (OC), serum total parathyroid hormone (PTH), insulin like growth factor 1 (IGF-1) and 25-hydroxyvitamin D (25-OH vitamin D) were recorded in the above study group preferably after an overnight fasting on the transfusion day.

After the recruitment and initial assessment, all patients were further referred to the Radiology Department for the assessment of bone mineral density values by DXA and QCT. All patients gave their informed consent to participate in the study. The referral Thalassaemia Unit is the reference centre for patients with thalassaemia for an area of about 1.000.000 inhabitants.

B. Methods

BMD of the total right hip, right hip neck, and lumbar spine (L1-L4) was determined by DXA in all patients. In case that the right hip was not eligible for scanning, the left hip was scanned instead. The available equipment was a Hologic Discovery QDR Series Densitometer (Hologic Inc, Bedford, MA), and all scans were performed with a standard technique according to the manufacturer's instructions. The device was calibrated daily in the morning with a coefficient of variation (CV) of 1% for the spine phantom. After each scan, images were analysed manually. In agreement with the latest official position of the International Society for Clinical Densitometry, only Z scores were calculated in our study group, because in females prior to menopause and in males younger than the age of 50, Z scores are preferred in BMD reporting. In such patients, a Z-score of -2.0 or lower is defined as "below the expected range for age".

Subsequently all patients underwent QCT examination by using a Toshiba (Tokyo, Europe) Aquilion 16-slice computed tomography scanner. All patients were scanned in the supine position, with a solid QCT phantom (Mindways Software Inc., Austin, TX, USA) placed below them, on the midline in the thoracolumbar region. The scanning protocol was the same for all patients. Scan parameters were 120 kV, 100 mAs, 1 mm (slice thickness), and 40 cm (field of view (FOV)). According to our quality control, this protocol has an in vitro coefficient of variation of 3.8 ± 2.2% for the phantom we use, which states in agreement with other researchers. After the scan, images were reviewed in the sagittal plane to identify any loss of vertebral height or wedge deformity, indicating an osteoporotic fracture. Then, 10 mm thick non-angled reconstructions were made through the centre of each T12-L3 vertebra. Trabecular BMD measurement was performed using a software package: QCT PRO 4.2.3. An oval region of interest (ROI) was placed in the trabecular bone at the anterior part of three vertebral bodies (L1–L3), excluding areas of sclerosis, the area of the basivertebral vein, and the vertebral cortex. A vertebral BMD of above 120 mg/cm^3 of hydroxyapatite was classified as normal, a vertebral BMD within 80–120 mg/cm^3 was classified as osteopenic, and a BMD below 80 mg/cm^3 was classified as osteoporotic. The patients did not receive any intravenous or oral contrast media three days prior to the study. None of the patients had pri-

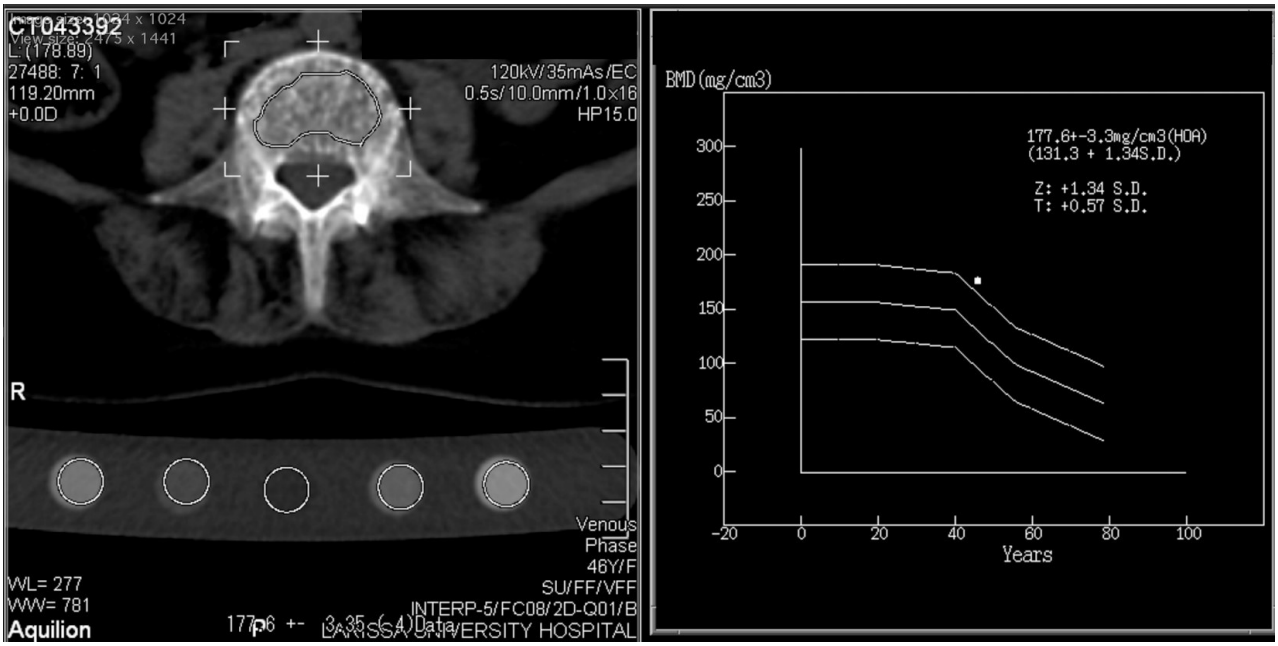

Fig. 1. *QCT measurement of an osteoporotic vertebral body in a patient with thalassaemia major. The BMD findings are ironically above normal.*

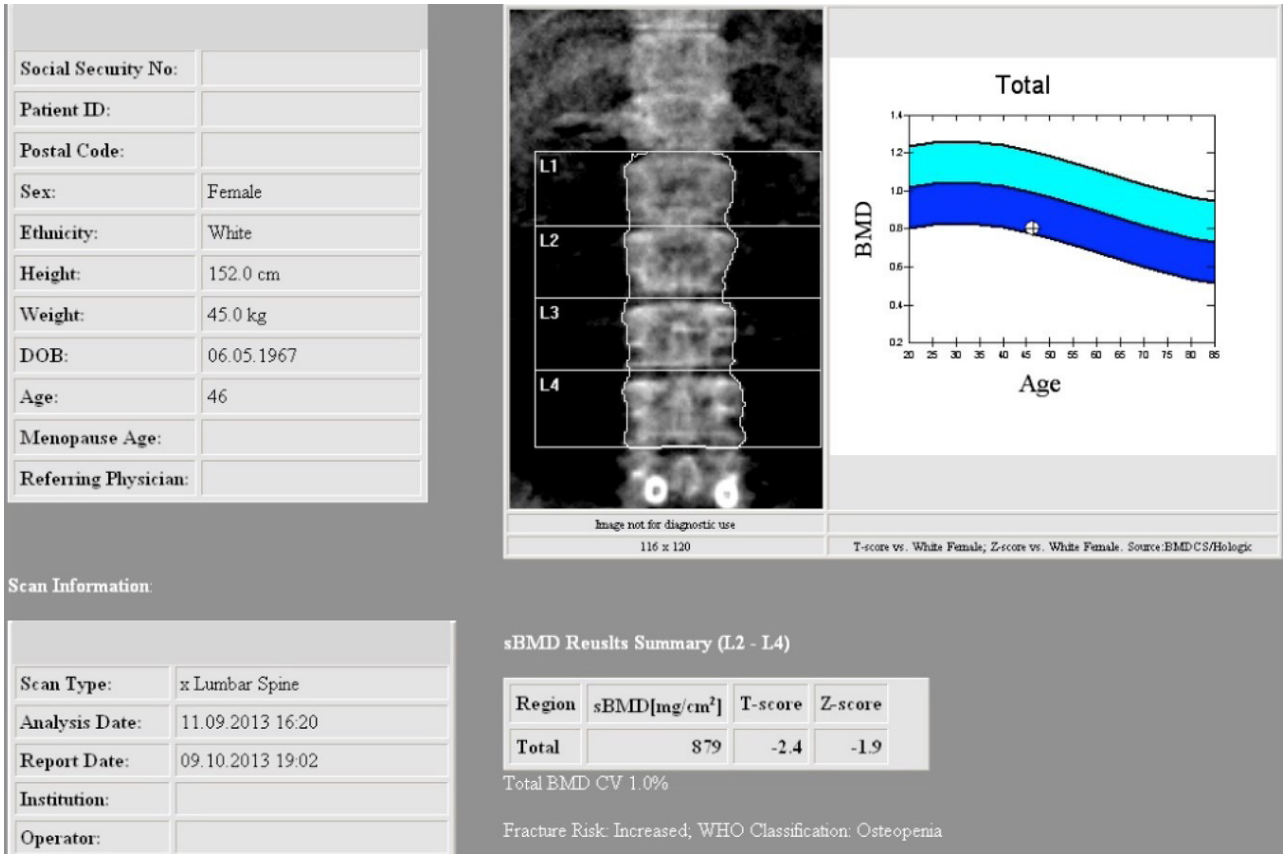

Fig. 2. *DXA measurements of the lumbar spine at the same patient, which show that BMD is diminished and the fracture risk is increased.*

or surgery with metal implants. As the reference data in the QCT machine was restricted to individuals older than 20 years, Z-scores were reported for patients older than this cut-off value.

C. Statistical Analysis

Characteristics of the group were presented by frequency distribution, for males and females separately. Comparison between groups was performed by Student's t-test or Wilcoxon rank-sum test. Pearson's correlation coefficient was calculated to evaluate the correlation between two variables. A p-value <0.05 was considered statistically significant. Analysis was performed on R statistical program, version 3.2.3.

Results

We examined 75 subjects, 31(42%) of which were men and 44(58%) were women. Their age was between 22 and 49 years (35±7.22) and 21 and 47 years (35.7±6.33), respectively. Men had BMI values which ranged between 16.1 and 30.4 (23±2.98) and women had BMI values that ranged between 17.2 and 33.2 (22.4±3.27). Similarly, their weight ranged between 53 and 94kg (68.7±10.1) and 44 and 92kg (61±10.4). Finally, their height was between 154 and 186cm (173±7.26) and between 150 and 177 cm (161±6.79), respectively. Laboratory characteristics are depicted in **Table 1**.

First, we examined the relationship of lumbar Z scores, at the level of L1, L2 and L3 vertebral bodies between DXA and QCT measurements. The correlation coefficients between DXA Z scores and QCT Z scores was found <0.1 (0.000000284 for females and 0.0102 for males). These values indicate no evidence of agreement between the two densitometric methods. QCT measurements expressed either normal or increased BMD values which reflected normal or increased Z scores, while measurements with DXA were far below normal. This was a paradox that led to the conclusion that QCT measurements were found to be unreliable in patients with thalassaemia.

DXA measurements of the lumbar spine as expressed by Z scores, detected 24/75 patients with osteoporosis (32%). The DXA measurements of the femoral neck as expressed by Z scores detected 10/75 patients with osteoporosis (10%).

Then, we analysed the correlation between DXA measurements and various bone turnover factors. We noticed a statistically significant positive correlation (p=0.03) between ferritin and DXA Z score of the femoral neck in the male group. We also found a statistically significant positive correlation (p=0.02) between osteocalcin and DXA Z score of the femoral neck in the male group. We also found a positive correlation (p=0.01) between ferritin and DXA Z score of L1-L4 vertebral bodies in the female group. Correlations between DXA Z scores, IGF-1 and vitamin D in both male and female groups showed no statistical significance.

Discussion

Patients with beta - thalassaemia major suffer from impaired bone metabolism that leads to decreased bone mineral density throughout the skeleton. Multiple factors contribute to this imbalance including nutritional malabsorption, dysfunction of endocrine glands due to iron deposition, reduced exercise, desferrioxamine toxicity and persistent bone-marrow expansion [1,2,9,10]. The prolonged life span of patients with thalassaemia major makes osteoporosis more prominent during adulthood, although studies in paediatric thalassaemic patients also suggest decreased bone mineral density. Despite improvement in treatment strategies over the last few decades, it has been estimated that 60 to 90% of adult patients with thalassaemia present with osteopenia or osteoporosis.

The increased risk of fracture can be prevented in patients with thalassaemia major if adequately recognized. However, the standard and advanced densitometric methods seem to work improperly among the above patients, because the iron overload that leads to liver and cardiac haemosiderosis creates a storage phenomenon into the bone marrow. Therefore, DXA measurements work relatively well but QCT measurements deviate substantially from the true bone mineral density of thalassaemic vertebral bodies [6-8].

Previous reports using dual x-ray absorptiometry (DXA) suggest that up to 70% of adults with thalassaemia major have low bone mass. Based on our findings, the percentage of patients with Z scores below -2.0 was 32%. This percentage, although high for a group of patients with a median age of 35 years compared to normal population, is considered relatively improved to other published data. The overall clinical care these patients receive during lifespan has been improved and therefore early bone loss has been prevented to some extent.

QCT has been reported as an unreliable method of

estimation of bone mineral status in thalassaemia major because it cannot discriminate between osseous minerals and iron [7]. The storage of iron in bone marrow, despite effective iron chelation, seems to be an important factor that renders QCT insensitive in bone mineral alterations. Our findings are consistent to those reported in previous studies and support the suggestion that QCT measurements can be falsely compared to DXA findings (**Fig 1**) (**Fig 2**). Therefore, QCT may be considered unreliable in detection of bone mineral alterations in patients with thalassaemia major.

However, bone demineralization occurs in patients with thalassaemia despite effective treatments. An important question is whether the fracture risk is underestimated with DXA measurements. A recent study implies that even the DXA method may suffer from inaccuracies leading to underestimation of osteoporosis in the above patients. Chelation therapy plays a protective role against demineralization of the skeleton; however, the multifactorial process against skeletal integrity renders osteopenia unavoidable during the lifespan of these patients [9,11].

Factors of bone turnover seem to be related with bone mineral density [11,12]. Our findings suggest that ferritin levels have a positive statistical correlation with Z scores of the spine in both male and female subgroups. Therefore, the higher the level of ferritin, the more likely the development of osteoporosis. Ferritin levels reflect the status of iron overload and are directly depended on chelation therapy [1,11,13,14,15]. Although thalassaemic patients include chelation therapy in their routine treatment, periodic or more permanent lack of compliance to the chelation regime, may contribute to haemosiderosis and impaired bone mineral density. Our findings also suggest a positive correlation between osteocalcin and osteoporosis that is evident among male patients. It may be suggested that osteocalcin plays the role of a biomarker in thalassaemia-induced osteoporosis in males, although our sample of patients is limited.

In conclusion, our findings support the following:

- DXA is the densitometric method that seemed to be more precise for detection and monitoring of bone alterations and subsequent osteopenia and osteoporosis among patients with thalassaemia major. The implementation of QCT in these patients is not reliable in terms of accurately measuring the bone mineral density and may lead to false interpretations.
- Osteoporosis is evident in a substantial percentage of adult patients with thalassaemia major. Factors that contribute to this suggestion include iron accumulation as expressed with high ferritin levels that reflect long-standing skeletal iron overload and alterations in osteocalcin that is more prominent in males.

Therefore, the findings of the present study indicate that the impaired bone metabolism in patients with thalassaemia major should be under medical monitoring and therapeutic prevention. **R**

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Ethical approval

Institutional Review Board, General Hospital of Larissa and University Hospital of Larissa, Greece.

Conflict of interest

The authors declared no conflicts of interest.

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