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Bone ultrasonometry, bone density, and turnover markers in type 1 Gaucher disease

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Abstract In 12 patients (mean age, 33 ± 13 years) with type 1 Gaucher disease (GD), we evaluated bone mass by broadband ultrasound attenuation (BUA) of the calcaneus and dual X-ray absorptiometry (DXA) of the total body, lumbar spine, and hip. In all patients, we measured serum levels of osteocalcin (OC) and bone-specific alkaline phosphatase (BAP) and urinary excretion of pyridinoline (Pyr/Cr) and deoxypyridinoline (D-Pyr/Cr) cross-links. Compared to age- and sex-matched healthy controls, patients with GD showed marked osteopenia at all measuring sites as expected. Values of BUA (67.25 ± 15.83 dB/MHz) were also significantly reduced. OC and BAP concentrations were within the normal range. Pyr/Cr and D-Pyr/Cr were significantly higher than in controls. Calculating T- and Z scores, we found a significant correlation between the Bone Severity Score Index (BSSI) and both BUA and BMD measurements. A significant correlation was also found between pyridinoline urinary excretion and both BSSI and BUA at the calcaneus. Our data suggest that type 1 GD in adulthood is associated with increased bone resorption and that BUA at the calcaneus may be a relevant tool in the assessment of bone status in these patients.

Key words Gaucher disease · biochemical markers · ultrasound densitometry

Introduction

Gaucher disease (GD) is an inherited metabolic disorder that results from defective activity of a lysosomal enzyme (acid beta-glucosidase, or glucocerebrosidase) required for the hydrolysis of glucosylceramide to glucose and ceramide [1,2]. This deficiency causes an accumulation of substrate in histiocytes and macrophages, which in turn leads to a multisystemic disease. Common presentation includes anemia, splenomegaly, cytopenia, and bone lesions. The manifestations of the disease are highly variable and are partially related to the size, number, and function of macrophages laden with glucosylceramide; however, the severity of bone disease generally does not correlate with the extent of visceral involvement nor with the degree of enzyme deficiency or concentration of circulating glucosylceramide [3,4].

Despite the relevance of skeleton abnormalities in almost all GD patients (who may show localized or diffuse bone loss, failure of remodeling characteristically occurring in the distal femur, osteosclerotic areas, and osteonecrosis), only a few metabolic and densitometric studies are available in the literature. We were able to find a single report on the measurement of bone mineral density (BMD) by dual-energy X-ray absorptiometry (DXA) in GD [5], and no report on the possible use of quantitative ultrasound (QUS) bone measurements, a new, radiation-free, and reliable technique that may give information not only on bone density but also on bone microarchitecture and elasticity [6–8]. As for bone turnover evaluation in GD, available data show some inconsistencies; although histomorphometric studies reveal abnormal bone architecture with loss of trabecular connectivity and increased number of osteoclasts [9], serum concentration of calcium, phosphate, osteocalcin, and immunoreactive parathyroid hormone are normal, as are urinary excretion of calcium and hydroxyproline [4].

The aim of the present study was to characterize bone metabolism in patients with GD using sensitive biochemical markers of bone turnover and bone density of the lumbar spine and femoral neck assessed by DXA. To assess the potential usefulness of bone ultrasound measurements in these patients, we evaluated the broadband ultrasound attenuation (BUA) of the calcaneus. BUA is an ultrasound (US) parameter that measures the loss of sound caused by bone as a function of frequency and appears to be the US parameter that shows the highest correlation index with BMD evaluated by DXA [10,11].

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To our knowledge, this is the first study comparing data on bone density, US parameters, and bone turnover markers simultaneously obtained in GD patients.

Subjects and methods

Patients

The study sample consisted of 12 Sicilian patients (8 men and 4 premenopausal women) with type 1 GD (mean age, 33 ± 13 years; range, 21–60 years) who were recruited after providing informed consent. The diagnosis of type 1 GD was based on the demonstration of marked deficiency of acid β-glucosidase activity in peripheral blood leukocytes. Four of 10 patients studied were heterozygotes for the N370S mutation; 2 other patients with mild clinical phenotype were homozygous for the I402T and V375L mutations, respectively. Patients had no history of major medical disorder affecting bone homeostasis (e.g., renal disease, hyperparathyroidism, malabsorption) nor were they taking medications with known effect on bone, such as estrogen, calcitonin, bisphosphonates, fluoride, oral corticosteroids, or calcium and vitamin D supplements.

The evaluation of the patients included bone scanning with technetium-99-labeled methylene diphosphonate and abdominal ultrasonography for imaging and measurement of the spleen and liver. The extent of organ involvement is expressed by the Severity Scoring Index (SSI) of Zimran et al. [12], which was used for assessment of the clinical phenotypes. Patients were assigned to the mild, moderate, or severe clinical phenotype based on a scoring index of 0–10, 11–25, and more than 25, respectively. A subscore (Bone Severity Score Index, BSSI) indicating the degree of skeletal involvement was calculated that ranged from absence of signs/symptoms to severe skeletal disease (presence/absence of chronic pain, fractures, joint replacement, and severe disability) [13]. Nine patients were splenectomized, and ten were receiving enzyme replacement therapy using a recombinant (imiglucerase) preparation, administered at 30–60 U/kg/month for a 2- to 8-year period.

Bone density measurements were performed by DXA of the total body, at the lumbar spine (L2–L4), and at the proximal femur (femoral neck), using a Norland XR-36 densitometer (Norland, Fort Atkinson, CO, USA). At these measurement sites, the precision of the method (coefficient of variation, CV) was 1.2%. The BMD measurements, expressed as area density in grams per square centimeter, were compared to sex- and age-matched healthy control subjects in a computer database.

BUA (dB/MHz) was measured at the left calcaneus using a dry US system (QUS-2; Metra Biosystem, Mountain View, CA, USA). The procedure includes calcaneal scanning, determination of the region of interest (ROI), and measurement of the ultrasound bone index (UBI), which is used to derive BUA. The QUS-2 can reproducibly scan and assess the same trabecular-rich ROI within and across individuals; this makes it possible to evaluate individuals with extreme foot size without the aid of a positioning device. The method has been described previously in detail by several study groups [14,15]. The precision of the method was determined by repeated measurements of a single individual to reach a 1.32% CV. The standardized CV (sCV) was 1.87 for BUA. Normative data drawn from 200 healthy Caucasian subjects in the age range of 21–79 years show a BUA value of 86.0 ± 14.3 dB/MHz.

In our patients, we measured biochemical indicators of bone formation and resorption. Serum for determination of calcium, albumin, phosphate, creatinine, osteocalcin, and bone-specific alkaline phosphatase (BAP) was obtained in the morning in the fasting state. Fasting urinary calcium, creatinine, pyridinoline, and deoxypyridinoline excretion was also determined. Serum calcium, phosphate, albumin and creatinine, and urinary calcium and creatinine levels were measured using standard laboratory methods. The ratio between urinary calcium and creatinine was given as mmol/mmol. Serum calcium was corrected for individual variations in serum albumin using this formula: corrected total serum calcium (mg/dl) = measured serum calcium + [(the normal mean albumin concentration − the observed albumin concentration) × 0.8]. BAP was measured by an enzyme-linked immunoabsorbent assay (Alkphase-B; Metra Biosystem). The sensitivity of the assay is 0.7 U/l; the intra- and interassay CVs are less than 10%. The normal reference range of BAP in adults is 15.0–41.3 U/l in men and 11.6–30.6 U/l in women. Osteocalcin level was determined by a competitive immunoassay (Novocalcin; Metra Biosystem). The sensitivity of the method is 0.45 ng/ml; the intra- and interassay CVs are less than 10%. The reference range in normal adults is 3.4–10 ng/ml.

Urinary free pyridinoline was measured using a competitive enzyme immunoassay (Pyrilinks; Metra Biosystem), and values are expressed relative to creatinine excretion (pyr/creatinine) and given as nmol/mmol. The intra- and interassay CVs are 7% and 11.2%, respectively. Normal values in healthy adults range from 12.8 to 25.6 nmol/mmol. Urinary free deoxypyridinoline measurement was performed using a competitive enzyme immunoassay (Pyrilinks-D; Metra Biosystem) that utilizes a monoclonal antibody with less than 2.5% crossreactivity with free pyridinoline. The intra- and interassay CVs are <10%. Values are corrected by urinary creatinine and expressed as nmom/mmol. Reference values in healthy controls range from 2.3 to 5.4 nmol/mmol.

Statistical analysis

The results of BMD were given as Z-score values (calculated as the difference between the actual measurement and the mean of healthy sex- and age-matched controls divided by their standard deviation). The results of BUA were given as T-score values. T scores were calculated as the difference between the actual measurement and the mean of healthy sex-matched young adults, as referred to the standard deviation of the sex-matched young controls. The results of biochemical indices of bone turnover were given as absolute values. One sample test statistic was used to check for significance concerning comparisons with normality. Nonparametric analysis (Spearman regression) was used to determine coefficients of correlation between variables. Statistical significance was taken as $P < 0.05$.

Results

The main clinical characteristics of GD patients are detailed in Table 1. Laboratory and technical data are given in Table 2. Values of densitometric variables (total body, lumbar spine, and femoral BMD, and calcaneal BUA) for the studied individuals were lower than the expected mean values ± 1 SD for healthy subjects as a function of age (Fig. 1). For the biochemical indices of turnover, the two markers of bone formation (BAP and osteocalcin) showed mean values of 30.08 ± 9.11 U/l and 7.56 ± 2.48 ng/ml, respectively, both within the range of normal values. Markers of bone resorption (Pyr/Cr; D-Pyr/Cr) showed mean values 28.63 ± 17.6 nmol/mmol and 6.13 ± 2.33, respectively. Serum concentrations of calcium and phosphate, and urinary excretion of calcium corrected by creatinine, were normal. Correlations between individual SSIs and individual T- and Z scores calculated for BUA and BMD, and between BUA, BMD, and biochemical markers, are reported in Table 3. QUS measurements were significantly correlated with BMD at the lumbar spine ($r = 0.59$; $P < 0.05$) and at the femoral neck ($r = 0.88$; $P < 0.001$). No statistically significant correlations were found between SSI and BUA or BMD at the various sites examined. SSI was not correlated with markers of bone turnover.

To evaluate better the impact of the degree of skeletal disease as judged by the clinical criteria and both densitometric and laboratory measurements, we correlated the individual BSSI with BUA, BMD, and bone turnover data obtained in serum and urine of GD patients. A stronger correlation was found between BSSI and BUA at the calcaneus ($r = -0.77$; $P < 001$). BSSI was also correlated with total, vertebral, and femoral BMD ($P < 0.05$), and with the urinary excretion of pyridinoline ($P < 0.05$).

Discussion

In the present study, patients with type 1 GD had reduced bone mass at the lumbar spine and at the femoral neck. This finding accounts for both trabecular (spine) and cortical (femoral neck) bone loss, and confirms previous findings by Pastores et al. [5]. BMD reduction might not be correlated with the degree and extent of visceral involvement, whereas there was a tendency for the most severe bone changes to occur in patients who had been splenectomized at a younger age. Moreover, it was not possible to relate the rate of osteopenia to genotype due to the wide genetic heterogeneity of these

Table 1. Main clinical findings in Gaucher disease (GD) type 1 patients

Patient no.	Age/sex (years)	Age at diagnosis (years)	Age at splenectomy (years)	Abnormal liver function	Other organ involvement (renal/lung)	Bone SSI[a]
1	23/F	1	2	−	−	3
2	25/M	4	4	−	−	10
3	27/F	19	22	−	−	1
4	34/F	25	25	+	−	1
5	37/M	30	5	+	+	3
6	38/F	30	np	−	−	1
7	38/M	35	3	−	−	16
8	40/M	34	np	−	−	1
9	47/M	44	44	+	−	1
10	50/M	41	np	−	−	1
11	60/M	59	np	−	−	3
12	60/M	60	20	−	−	18

−/+, absent/present; np, not performed

[a] Bone Severity Score Index (SSI) ≥ 10 indicates severe bone involvement (chronic pain, fractures and/or joint replacement, severe disability)

Table 2. Bone ultrasound measurements, bone mineral density, and markers of bone turnover in 12 patients with type 1 GD

Patient no.	Total body BMD Z score	Lumbar spine BMD Z score	Femoral neck BMD Z score	BUA T score	BAP (U/l)	OC (ng/ml)	Upyr/Cr (nmol/mol)	Ud-pyr/Cr (nmol/mmol)
1	−1.62	−1.75	−0.60	−2.00	31.49	6.21	44.60	9.87
2	−3.36	−3.10	−2.70	−2.34	41.12	10.72	33.87	7.96
3	−2.25	−1.90	−1.72	−1.88	42.6	5.22	25.82	5.64
4	−2.37	−0.40	−0.96	−0.61	16.68	8.68	12.15	4.69
5	−3.36	−3.30	−0.71	−1.81	39.26	11.63	30.02	6.24
6	−1.95	−2.03	−1.97	−2.15	15.67	6.98	14.11	4.19
7	−3.02	−2.84	−2.78	−3.21	37.66	8.72	31.22	6.58
8	−2.12	−3.10	−1.54	−1.41	26.08	10.41	21.14	4.51
9	−2.62	−2.00	−0.60	−0.41	32.92	3.44	24.94	5.52
10	1.007	0.98	0.78	1.13	22.46	6.12	14.22	4.38
11	−1.90	−3–30	−2–12	−2.73	25.49	5.76	15.85	3.26
12	−4.25	−3.25	−3.00	−3.56	29.54	6.84	75.60	10.80
Mean ± SD	−2.318 ± 1.29	−2.166 ± 1.32	−1.493 ± 1.11	−1.748 ± 1.29	30.08 ± 9.11	7.56 ± 2.48	28.63 ± 17.6	6.13 ± 2.33

BUA, broadband ultrasound attenuation at calcaneus; BMD, bone mineral density; BAP, bone-specific alkaline phosphatase; OC, osteocalcin; Upyr/Cr, urinary pyridinoline/creatinine excretion; Ud-pyr/Cr, urinary deoxypyridinoline/creatinine excretion

Fig. 1. Bone density in patients with Gaucher disease at different measuring sites expressed as Z score (total, spine, and neck) and as T score (broadband ultrasound attenuation, BUA)

patients, which reflects the polyracial composition of the Sicilian population.

BUA measurements at the calcaneus also showed low BUA T-score values. As the os calcis is composed almost entirely of cancellous bone, with more than 90% of trabecular bone by volume, measurements at this site may give additional information about trabecular bone loss in these patients. Most clinical US studies performed in patients with postmenopausal osteoporosis [6], chronic renal failure [16], primary hyperparathyroidism [17], and anorexia nervosa [18], as well as in normal subjects [19], show significant correlations between US parameters and BMD at various skeletal sites. We should, however, note that it is difficult to characterize patients with GD as a group: the extent of bone involvement is variable, and although osteopenia is a common finding in GD, the lack of uniformity in the types of bone lesions does not allow us to predict the overall severity of skeletal involvement by bone density measurements made at a single anatomic site. In this respect, we should keep in mind that a number of studies conducted to characterize the properties of bone that are measured by QUS have demonstrated a difference between QUS and BMD, suggesting that QUS techniques may provide additional information concerning the structural properties of bone that are not captured by BMD. In particular, ultrasound variables such as BUA depend more on skeletal geometry (microarchitecture, connectivity, porosity) than merely on bone calcification. It is therefore possible that US measurements are most reliable in assessing the skeletal impact of GD in its various stages.

The pattern of the markers of bone turnover seems to indicate an increased bone resorption in our patients: we found that both pyridinoline and deoxypyridinoline urinary excretion were significantly increased as compared with controls. On the contrary, the two markers of bone formation (i.e., serum osteocalcin and BALP)

Table 3. Correlations (Spearman *r*) between Severity Score Index (SSI), Bone Involvement Index (BSSI), bone densitometric parameters, and markers of bone turnover in 12 patients with type 1 GD

	SSI	BSSI	BUA	BMD^{TB}	BMD^{LS}	BMD^{FM}
BUA	−0.52	−0.77**	1	0.37	0.59*	−0.88**
BMD^{TB}	−0.41	−0.67*	0.37	1	0.50	0.52
BMD^{LS}	−0.42	−0.68*	0.59*	0.50	1	0.58*
BMD^{FN}	0.54	−0.70*	0.88**	0.52	0.58*	1
BAP	0.07	0.33	−0.18	−0.52	−0.24*	−0.15
OC	0.04	0.39	−0.18	−0.45	−0.40	−0.26
Upyr/Cr	0.26	0.59*	−0.55**	−0.51	−0.34	−0.34
Ud-pyr/Cr	0.16	0.49	−0.39	−0.61*	−0.10	−0.25
SSI	1	0.81**	−0.52	−0.41	−0.42	0.54

BUA, broadband ultrasound attenuation at calcaneus; BMD^{TB}, total body bone mineral density; BMD^{LS}, lumbar spine bone mineral density; BMD^{FN}, femoral neck bone mineral density; BAP, serum bone-specific alkaline phosphatase; OC, serum osteocalcin; Upyr/Cr, urinary pyridinoline/creatinine; Ud-pyr, urinary deoxypyridinoline/creatinine
$P < 0.05$; $P < 0.001$

measured in this study do not seem relevant, as their concentrations remain within the range of normal values.

Drawbacks of the present study are the small group of patients studied and the absence of histomorphometric data. These potential limitations, however, should not negate the finding that QUS measurements are decreased in GD patients, suggesting an important application of US techniques in estimating bone health in GD. The observation of a different relationship between BUA and BSSI and between BMD and BSSI would support, although tenuously, the hypothesis that calcaneal BUA can give information on bone complementary to BMD measured by DXA at the spine and femoral neck.

Further studies are required to asses the clinical relevance of this finding in the management of GD; however, some advantages of QUS devices (lower cost, portability, and the absence of radiation exposure) would encourage their use in the evaluation of GD patients.

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