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Evaluation of Bone Mineral Quality by Phalangeal Quantitative Ultrasound in Perinatally HIV-Infected Youths

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ABSTRACT

Decreased bone mass density (BMD) is now recognized as an emerging metabolic complication of HIV infection. Several studies have well described the elevated bone turnover in BMD among HIV-infected people. Quantitative ultrasound (QUS) assesses skeletal status by measuring the amplitude-dependent speed of sound (AD-SoS, m/s) and the bone transmission time (BTT, μs). This technique is safe, easy to use, radiation-free and "friendly," for these characteristics it is particularly indicated among children. The aim of the present study is to evaluate the effects of HIV infection on bone quality by phalangeal QUS in a young population of perinatally HIV-Infected children: 44 patients (23 females, 21 males; aged 3-17 years) were examined and compared with a control population (1227 healty children, 641 males and 586 females; aged 3-16 years). All patients were vertically infected, 7 patients were CDC stage C, 18 B, and 18 A; considering the antiretroviral treatments 4 were naive to any therapy, 7 were taking two NRTIs, and 32 were on HAART. QUS values were significantly lower in cases than in controls, even after adjustment for age and body size. The associations of AD-SoS and BTT with age, skeletal age SDS, height, and therapy duration were statistically significant. Gender, type of therapy, and CDC stages were not associated to AD-SoS and BTT. This article suggests that QUS measurements could be an attractive option for the evaluation of bone quality in HIV-infected children.

Introduction

Different diseases may directly or indirectly decrease bone mineral content, and osteoporosis and osteopenia are now recognized as emerging metabolic complications of HIV infection. For this reason, bone mineral density (BMD) could be an important parameter to evaluate in choosing the right therapeutic strategy and best follow-up in HIV-infected patients with metabolic bone diseases. It is well known that 90% of adult bone mass is gained in the first two decades of life, and that a low peak bone mass at the end of adolescence may accelerate the development of osteopenia and osteoporosis during life. Environmental factors (adequate nutrition and physical activity) and genetic factors play a crucial role in determining whether or not children acquire an appropriate amount of bone mass for their body size. Several studies have reported an increased prevalence of reduced BMD among HIV-infected subjects (1-12), including those who have not received antiretroviral drugs. Bone loss in these patients probably has a multifactorial origin, including HIV bone cell infection, viral load, highly active antiretroviral therapy (HAART), inflammatory cytokine effects on osteoblast and osteoclast activity, malnutrition, malabsorption, reduced calcium intake, decreased physical activity, and hormonal disorders (13-19). Some densitometric techniques to assess bone mineral status developed for adults have been adapted for use in children. Dual energy X-ray absorptiometry (DXA) is the most commonly used technique for bone health assessment, being relatively low cost with short scanning times and minimal radiation exposure (less than 13μSv, annual radiation exposure limits recommended by the National Council for Radiation protection (20) for public infrequent exposures and limits recommended by Federal Drug Administration Regulation for exposure from medical research procedures in children are both 5000μSv).

The major disadvantage of DXA is the two-dimensional evaluation of areal BMD (aBMD) that cannot give a true measure of volumetric BMD (although mathematical methods have been proposed to reflect BMD more closely). Quantitative ultrasound (QUS) is a relatively recent non-invasive method of estimating bone mineral status at the peripheral skeleton. The phalangeal QUS method is based on the transmission of ultrasound (US) through the distal diaphysis of the proximal phalanx of the dominant hand. The graphic tracing obtained reflects the characteristics of the electrical signal generated by US after crossing the phalanx soft tissues and bone. In

addition to BMD assessment, QUS also provides some structural data which may be important in determining fracture risk (21-22). QUS has several advantages: firstly, it is can be performed with a portable device and is technically simpler, more economical and friendly than DXA; secondly, it is radiation-free. These characteristics make QUS particularly indicated for assessment of bone mineral quality among children and adolescents. Despite its proven advantages, QUS utilization remains controversial because of poor knowledge on the physical mechanism of ultrasound in assessing bone characteristics, technological diversity among QUS devices, use of different QUS variables to estimate bone mineral status, and the difficulty of comparing the results obtained by QUS with those acquired by DXA. Recent studies have clarified most of these aspects leading to the clinical application of QUS methods in a large number of disorders, including HIV infection. The aim of this study was to evaluate a population of HIV-vertically infected children with QUS technology to determine the effects of disease and therapy-associated factors on bone mass (BM).

Methods

We studied BM in a cohort of 44 HIV-perinatally infected children and adolescents (21 males and 23 females) followed regularly at the Infectious Diseases Clinic, University of Genoa. All patients, aged three to 17 years were screened and consecutively enrolled between February 2001 and August 2002. The parents of the study participants provided written informed consent. The diagnosis of HIV infection and the clinical and immunological status of the patients met the criteria of the 1994 revised Centers for Disease Control and Prevention (CDC) HIV classification for children (23). None of the enrolled patients had severe concomitant diseases, hand deformities or recent fractures, and none of them had ever been on pharmacologic therapy with recombinant human growth hormone (rH-GH) or steroids, or needed calcium or vitamin supplements. The control group comprised 1227 healthy children (641 males and 586 females) aged three to 16 years recruited from three schools in Genoa. Non-caucasian children were excluded from this study for the well-known ethnic difference in BMD (24).
The study participants were evaluated at the study entry, and history-taking included data on age, sex, CDC clinical staging, and current antiretroviral therapy. Type of antiretroviral therapy and duration of all therapies were abstracted from our clinical recordings. At the time of bone assessment seven out of 44 patients (9%) were naive and 33 out of 44 (75%) were on HAART.
We performed anthropometric measurements according to standard techniques (25): height was measured with a Harpenden stadiometer, weight with a standardized scale and then we calculated Body Mass Index (BMI). Height and weight were expressed as continuous variables in SDS units, determined as the difference between the individual observed value and the reference mean for age and sex, divided by the corresponding standard

deviation. Height reference charts of Tanner and Whitehouse and BMI reference charts of Rolland-Cachera et al. were used (26-27).
Skeletal age was determined using the Greulich-Pyle method, since the Italian population proved to fit these standards very well (28). The difference between skeletal and chronological age was calculated and expressed as SDS adjusted for age and sex.
A blood collection was taken for T lymphocyte count (CD4+ and CD8+ percentage and count). Plasma HIV-RNA viral load was measured by a commercial quantitative reverse transcriptase polymerase chain reaction kit (AMPLICOR HIV Monitor test; Roche Molecular Systems, Branchburg, NY) with a lower detection level of <50 copies/ml.
BMD was evaluated with a phalangeal-QUS device (DBM Sonic BP IGEA). Two main variables can be measured by this technique:

1. Amplitude-dependent speed of sound (AD-SoS, m/s) is derived by measuring the interval between the start time of the transmitted signal and the time the signal received reaches the predetermined minimum amplitude value of 2 mV for the first time (29-30). This variable is influenced by structural characteristics of growing bone and by bone mass and size (31).

2. Bone transmission time (BTT, μs) represents the difference between transmission time in phalanx soft tissue and bone and transmission time in phalanx soft tissue, and hence reflects the bone properties independent of the confounding effect of soft tissue (32).

AD-SoS and BTT give information on bone mineralization and BM; these measurements were performed by the same single observer (repetitively as root mean square coefficient of variation was 0.81 for AD-SoS and 3.12 for BTT, respectively). Calibration and time stability of the device were checked daily with an internal calibration procedure. AD-SoS and BTT SDS were calculated as the difference between the individual observed value and the mean value for age and sex determined in the reference population, divided by the corresponding standard deviation (SD) (33). The statistical analysis was carried out using the Statistical Package for the Social Sciences (SPSS) version 11.0 for Windows. Comparisons between groups and multivariate analyses were carried out using an ANOVA model. Correlations were evaluated using the Pearson correlation coefficient.

Results

We enrolled 44 HIV-vertically infected patients, 21 boys and 23 girls; mean age was 10.4 years (median: 10.7; range: 3.6-17.1). All had symptomatic disease according to the CDC classification: 18 A, 18 B and eight C. They had the following immunovirological characteristics on enrolment: median CD4+ T cell count were 630 cells/mm³ and 27.2% (range: 13-2776 cells/mm³ and 2.6-46.6%, respectively); median CD8+ T cell count and percentage were 973.5 cells/mm³ and

39% respectively (range: 98-2913 cells/mm³ and 19-75.1%); median viral load was 1,980 HIV-RNA copies/ml (range: <50-818,000). Basic auxological and QUS variables of the patients are reported in Table 1.

HIV-infected patients, especially males, showed a tendency towards short stature, as shown by a mean height SDS about 1 SD below the median reference value. Mean BMI SDS was negative by 1.7-1.9 standard deviations in both sexes. Skeletal age was significantly lower than chronological age (mean standardized difference= -0.87, p < 0.001) and this difference was more pronounced in boys (-1.4 vs. -0.4; p = 0.04). Skeletal age SDSs as a function of chronological age and fitted linear regression curves,in males and in females separately are reported in Figure 1.

In patients, mean AD-SoS and BTT were 1879.7 (SD=57.2) and 0.80 (SD=0.32) respectively. These values were significantly lower (p<0.001 for both variables) than the means of the reference population, being 1924.7 (SD=64.9) for Ad-SoS and 0.97 (SD=0.3) for BTT. The associations between AD-SoS and BTT with other variables [sex, type of therapy (HAART vs no HAART), protease inhibitors (PIs)-containing therapy (yes vs. no), CDC stage)] were not significant (Table 2). Significant associations were found for both AD-SoS and BTT with chronological age, skeletal age and skeletal age-SDS, height and therapy duration (Table 3).

Patients' AD-SoS and BTT are reported in Figure 2 as a function of chronological age in relation to normative curves obtained in the reference population. Almost all AD-SoS and BTT fell below mean reference values.

To test whether the difference in AD-SoS and BTT between pts and controls could be due to skeletal age delay, two multivariate regression models with AD-SoS and BTT as dependent variables respectively, including age, skeletal age SDS, height, BMI and disease state (affected vs. controls) were fitted. For both AD-SoS and BTT, the disease state was significant (p<0.0001) after correction for all the above variables. The estimated AD-SoS and BTT decrease in diseased patients after adjusting for all the other variables included in the model was on the average 31.4 (SE=8.6) and 0.086 (SE=0.035).

Discusssion

Osteoporosis and osteopenia have been reported among children and adults with HIV infection (34,35). The role of QUS methods in the diagnosis of a reduced mineral status in HIV-infected children should be considered similar to that of DXA (22). Low values of QUS parameters were found in this group of patients compared with the reference population. Few studies in the literature used QUS to evaluate bone mass in HIV-infected patients, especially in children and adults. Like the majority of the reports measuring bone mass with DXA technology, our study confirmed the tendency toward osteopenia and osteoporosis in these patients. DXA may also be used to identify HIV-infected children who could be exposed to an increased risk of osteoporosis in adulthood, but the exposure to ionizing radiation is a limiting factor for preventive studies in large populations of children.

BM measurements in growing subjects must necessarily be interpreted by taking into account size and skeletal maturity, since height, weight and skeletal age are powerful determinants of bone mass. The HIV-infected boys included in the present study showed mild growth retardation, while the girls' height and skeletal age were more appropriate for age. Low BMI was found in both sexes, possibly indicating a poor nutritional state and/or lower amounts of lean body mass. Skeletal age was significantly lower than chronological age, and several patterns of disturbed growth have been reported in as many as 50% of HIV-vertically infected children and adolescents (36-37). Pubertal development stages were not considered, since they may be used as a biological maturity measure only during the pubertal period.

The pathogenic mechanism of BM loss has not fully been elucidated. Chronic inflammation caused by HIV infection has been associated with

TABLE 1. Characteristic of the study population

VARIABLES	Male (Mean SD)	Female (Mean SD)
Height (cm)	130.0 (22.4)	136.6 (21.4)
Height-SDS	-1.09 (1.23)	-0.6 (1.36)
Weight (Kg)	30.6 (16.7)	34.7 (13.0)
BMI (Kg /m2)	16.1 (4.7)	16.4 (5.4)
BMI-SDS	-1.94 (1.63)	-1.74 (1.31)
Skeletal age (years)	8.7 (4.4)	10.4 (3.8)
Skeletal age SDS	-1.4 (1.4)	-0.4 (1.3)
AD-SoS (m/sec)	1867.6 (50.9)	1890.7 (61.5)
AD- SoS-SDS	-1.94 (1.62)	-1.74 (1.32)
BTT (µ)	0.76 (0.32)	0.83 (0.32)
BTT-SDS	-1.12 (1.34)	-0.75 (1.16)

bone absorption (38), and HIV itself may exert a direct effect on bone metabolism. Hormones, cytokines and body composition contribute to low BMD in HIV-infected patients (11,12,39-41). Bone is constantly undergoing remodelling in a synchronized balance between absorption and formation, which can become unregulated during HIV-infection (1).

Disease-related processes might act in many ways. Low CD4+ and a high level of IL-6 might influence bone loss as this cytokine activates osteoclasts and appears to interfere negatively with IGF-1 secretion or activity (17). In addition, HIV-infected children may have a reduced intake and/or absorption of available calcium for bone mineralization, together with proteins (18, 42).

Other important pathophysiologic mechanisms underlying elevated bone turnover have been partially clarified. In healthy people, osteoblasts express receptors for activation of nuclear factor kappa β ligand (RANKL) which interacts with a cell surface protein, called RANK, found on osteoclast precursors, thereby inducing differentiation and proliferation of osteoclasts. Osteoblasts also secrete osteoprotegerin (OPG) which binds to RANKL and prevents osteoclast activation. Activated T cells also express RANKL, so that HIV-infected patients present elevated serum RANKL concentrations associated with lower BMD (43). These patients also present elevated OPG concentrations, which may represent a compensatory mechanism to reduce osteoclast activation and excessive bone resorption (44). We did not measure biochemical or cytokine serum levels in this study, precluding any conclusions on the pathophysiologic mechanisms mentioned above.

The present study did not indicate a clear-cut role of antiretroviral therapy as a risk factor for a decreased bone mass in HIV-infected patients. Many other studies showed various correlations between HAART, PI-based HAART, and lower BMD: patients on HAART or on a PI-based regimen did not have significantly lower BMD than patients naive or never treated with PIs (45). The good immunovirological status of our patients, with concomitant clinical benefit to most of them, may suggest that HIV per se, and/or other factors related to the natural evolution of the disease play a crucial role in determining an early presentation of osteopenic syndromes. However, like the majority of HIV-infected patients on HAART, most of the population in this study changed various antiretroviral drugs during their longitudinal follow-up because of virological failure and/or side effects. This limitation in defining the exact effects of single antiretrovirals on BM, prevents further evaluation on the impact of specific classes of antiretrovirals.

In conclusion, it is important to be aware of the possibility of bone remodelling changes in all patients and long-term prospective longitudinal studies are required to investigate bone mass and metabolism, growth and skeletal maturation, lipodystrophy, hormonal growth factors and immune system relationships, nutritional intake, calcium balance, and the effects of antiretroviral medications.

On the basis of our experience, a non-invasive technique like QUS technology opens up interesting new perspectives, allowing bone mass changes to be monitored in vertically infected patients for a better evaluation of the role of disease-related conditions or treatments able to interfere with bone mass acquisition during growth.

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