Original Article

Quantitative ultrasonometry: An alternative and easy method to evaluate bone quality in people living with human immunodeficiency virus

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Abstract

Background: HIV infection and antiretroviral therapy (ART) are associated with bone mineral loss. DXA is the gold standard method to evaluate the status of bone mineral density (BMD). However, it is not always readily available. An easy method is needed to evaluate bone quality in those infected with HIV. **Objective:** To evaluate portable quantitative ultrasonometry (QUS) as an alternative technique to provide information about bone density, bone strength, and the bone turnover markers in HIV-infected people. **Methods:** A total of 69 men took part (34 HIV-infected men were matched with 35 non-HIV-infected men) in the study. Bone mineral status was assessed by the Achilles quantitative ultrasonometer at the calcaneal heel. The HIV status was recorded for all HIV-infected patients. Calcium-regulating hormones and bone turnover markers were assessed in all participants. Results: The mean age was 47.8±7.8 years and 49.1±6.00 years for the HIV-infected and non-infected population, respectively. The bone quality expressed as Stiffness index (SI) was reduced in HIV-infected patients. Bone turnover markers were higher in the HIV-infected patients, P1NP (ng/mL) was 48.0±14.3 vs 41.1±15.2 (P=0.057), and the (CTx)) (ng/mL) was 0.41±0.18 vs 0.29±0.11 (P=0.002). **Conclusions:** QUS is easy to use. Hence, QUS could be used as alternative method for screening of HIV patients for altered bone status.

Keywords: HIV, Bone Mineral Density, Achilles Quantitative Ultrasonometer

Introduction

The use of highly active antiretroviral therapy (HAART) drugs since 1996 has dramatically reduced morbidity and mortality of human immunodeficiency virus (HIV)-infected individuals. Today, HIV-infected persons have a life expectancy that is similar to that of non-HIV-infected persons[1,2]. However, disorders that typically affect the aged population now appear in relatively young HIV-infected individuals, including HIV-associated neurocognitive disorder (HAND), cardiovascular disease (CVD), metabolic syndrome (MS) and non-HIV-associated malignancies and bone abnormalities[3].

Low bone mineral density (BMD) is an emerging metabolic condition in HIV-infected patients, with an estimated incidence that is three-fold greater than in the general population[4]. Many factors contribute to loss of BMD in HIV-infected patients, such as smoking, alcohol use, effects of long-term HIV infection, persistent of immune dysfunction and HAART-induced toxicities[5].

Regular evaluation of BMD in people living with HIV (PLWH) is a must priority. Different country specific guidelines are used to monitor and follow BMD in PLWH. These guidelines are similar to the general population recommendation of using screening using dual energy X-ray absorptiometry (DXA) in PLWH, in women who are postmenopausal and men aged at least 50 years[6,7]. In spite of these guidelines, clinical practice approaches to screening may vary because of different factors, including access and availability of necessary resources for screening and treatment. Lack of access to DXA in developing countries and regulatory

restrictions may be a major barrier to screening and future management[8].

A number of studies[9,10] have shown that quantitative ultrasonometry (QUS) is a useful tool for fracture risk prediction in different populations and conditions, but to the best of our knowledge, there is only one publication on HIV for Senegalese patients[11]. Additionally, QUS is radiation-free, portable, inexpensive and may provide reliable measures of changes in bone quality, having been proven to predict hip fractures and osteoporotic fractures in Caucasian postmenopausal women and elderly men[12-14].

QUS measures both velocity and amplitude properties of ultrasound waves through bone tissue. The velocity of the measured waves, speed of sound (SoS) and broadband ultrasound attenuation (BUA) are the most commonly used measures to assess bone tissue as well as values calculated from a combination of these two, the stiffness index (SI) and quantitative ultrasound index (QUI) prediction[15,16].

The first objective of our study was to compare bone status measured by the QUS between HIV-infected men followed at the HIV clinic in the Rambam Medical Centre in Haifa, Israel with age-matched control subjects. The second objective was to assess the utility of QUS expressed as Stiffness index (SI) in HIV-infected patients in relation with bone turnover markers procollagen type 1 N-terminal propeptide (P1NP) and C-terminal telopeptide of type 1 collagen (CTx) compared to controls. The third objective was to determine influence of HIV-related traditional factors on bone quality in PLWH compared with sex-age matched control studies

Materials and methods

The observational study enrolled HIV-infected and non-HIV-infected men from similar demographic backgrounds. The HIV-infected men were recruited from the HIV clinic in the Rambam Health Care Centre in Israel and non-HIV-infected men from the local area.

After signing informed consents for participation in the study, a detailed medical history was obtained from all participants with emphasis on skeletal pain sites, previous fractures and factors affecting bone metabolism. Physical examination included measuring age, weight, height and body mass index (BMI).

Bone quality was measured at the calcaneus with the Achilles Express ultrason ometer (GE Lunar, Madison, WI). HIV status for HIV patients (CD4 count and viral load at diagnosis, at start of HAART, nadir and current at recruitment and present and past HAART). The results expressed as T scores of stiffness index (SI) – a composite of Speed of Sound (SOS) and Broadband Ultrasound Attenuation (BUA).

Laboratory evaluation

Routine laboratory tests included serum calcium, phosphate, albumin, creatinine, alkaline phosphatase, ALT, AST and Mg. Calcium-regulating hormones and bone turnover markers were also assessed, including

Table 1. Demographic characteristics of the patient groups.

	Infected, n = 34	Non-infected, n = 35	P value
Age at examination (y)	47.8±7.8	49.1±6.00	0.44
BMI	26.1±3.2	27.5±4.7	0.16
Smoking Current Never Past	14 (41.2%) 13 (38.2%) 7 (20.6%)	11 (31.4%) 17 (48.6%) 7 (20.0%)	0.64
Alcohol Daily None Occasionally	1 (2.9%) 13 (38.2%) 20 (58.8%)	0 15 (42.9%) 20 (57.1%)	0.57
Physical activity 1–4 times per week Every day None	17 (50.0%) 1 (2.9%) 16 (47.1%)	22 (62.9%) 0 13 (37.1%)	0.38
Fractures in the past (y)	7 (20.6%)	7 (20.0%)	1.00
Maternal history fractures (y)	9 (26.5%)	4 (11.4%)	0.13
Any supplements diagnoses (y)	5 (14.7%)	9 (25.7%)	0.37
Concomitant medication (y)	7 (20.6%)	11 (31.4%)	0.41
HIV transmission risk group, n (%) Heterosexual Homosexual Intravenous drug users	11(32%) 14 (41%) 9 (27%)		
Total HAART exposure <1 year 3–5 years >5 years	8 (23.5%) 7 (20.5%) 19 (56%)		
TDF, n (%)	25 (86%)		
Nadir CD4$^+$ cells/mm^3 [median 25–75%]	247 [141–334]		
Current CD4$^+$ cells/mm^3 [median 25–75%]	510 [352–753]		
HIV RNA < 20 copies/mL, n (%)	32 (94.1%)		

PTH, serum 25(OH) D$_3$ as well as bone turnover markers P1NP and CTx. The inclusion criteria for HIV patients and controls were age between 35 and 60 years old, more than 5 years after HIV diagnosis and being on HAART. The exclusion criteria for both groups were past diagnosis and/or treatment of osteopenia/osteoporosis, previous diagnosis of any condition requiring steroid treatment, diagnosis of primary hypoparathyroidism or primary hyperparathyroidism, past or present malignancy, inflammatory bowel disease and any kind of malabsorption disorder.

Table 2. QUS measured BMD (T-scores) and laboratory data of the two study groups.

	HIV positive n = 34 Median (range)	HIV negative n = 35 Median (range)	P value
Calcaneus SI T-score	−0.65 (−1.13 to −0.5)	0.3 (−0.5 to 0.9)	0.026
Serum 25 (OH) D3 (ng/mL) **Vit. D <30** **Vit. D ≥30**	19 (56%) 15 (44%)	25 (71%) 10 (29%)	0.22
PTH (ng/L)	44.1±15.9	40.5±13.5	0.33
CTx (ng/mL)	0.38±0.18	0.28±0.11	**0.008**
P1NP (ng/ml)	45.1±14.3	40.1±15.2	0.015
Alkaline phosphatase (U/L)	74 [42–173]	70 [51–137]	0.38
Calcium (mg/dL)	9.2 [8–10]	9.3 [9–10]	0.13
Phosphorus (mg/dL) (inorganic phosphate)	3.3 [2.7–5.3]	3.2 [2.3–4.1]	0.28
Albumin (g/dL)	4.10 [3–5]	4.2 [4–5]	0.79

Table 3-A. Univariate analysis for predicting SI T-score $\leqq 1$ in the study population.

	Odds ratio	P value	95% Confidence interval
Age at exam	1.02	0.6	0.95–1.10
BMI	1.02	0.71	0.90–1.16
Smoke **present vs. past**	1.38	0.55	0.48–3.92
25 (OH) vitamin D$_3$ **≥30 vs. >30**	2.67	0.069	0.93–7.70
Infected/non-infected	3.82	0.018	1.26–11.6
Alcohol **Occasionally/Never**	0.66	0.43	0.23–1.86
Physical activity **Yes/No**	0.41	0.096	0.14–1.17
History of fractures **Yes/No**	1.35	0.63	0.39–4.67

Table 3-B. Multivariate analysis in the study population.

	Odds ratio	P value	95% Confidence interval
Age at exam	1.04	0.37	0.96–1.13
BMI	1.10	0.26	0.93–1.31
Smoke **Past + current** **Past (ref)**	1.18	0.81	0.30–4.56
Vit. D cut ≥30 ng/ml	2.67	0.069	0.93–7.70
Vit. D <30 ng/ml ref)	3.51	0.07	0.90–13.71
HIV Infected **Non HIV Infected**	5.35	0.012	1.44–19.78
Alcohol (yes)	0.93	0.92	0.26–3.35
Physical activity (yes)	0.55	0.38	0.14–2.10
Fractures in the past (yes)	0.95	0.95	0.19–4.56

Statistical analysis

Descriptive statistics on terms of mean, STD, median, percentiles and ranges were presented for all parameters in the study. Normal distributions of the quantitative parameters were tested by Kolmogorov-Smirnov test. As a result of this test, parametric and non-parametric tests were used for differences between groups. Differences between the two groups (infected vs. non-infected) were tested by T-test, Mann Whitney U test and Fisher exact test. Furthermore, odds ratio (OR) and 95% confidence interval (CI) were also used for analysis between two groups. Multivariate logistic regression model for predicting T-score $\leqq 1$ was used to determine the effect of the independent parameters. P<0.05 was considered statistically significant. SPSS software version 25 was used for the statistical analysis.

Results

Study population

We enrolled 69 men, 34 (49%) HIV-infected participants received follow-up at the AIDS clinic at the Rambam Medical Centre in Haifa, Israel and 35 (51%) non-HIV-infected men from the local area (Table 1). All of the HIV-infected people were receiving antiretroviral treatment and were in good immunological condition (median CD4=510 cell/mm^3). The majority (32 of 34) of HIV-infected individuals had undetectable viral loads.

We found a statistically significant decrease in SI (expressed in T-scores, P = 0.026) and increase in bone turnover markers (P1NP and CTx) in the HIV-infected patients compared with the healthy controls (P=0.008

Table 4. Univariate analysis for predicting T-score ≤ −1 in HIV infected patients.

	Odds ratio	P value	95% Confidence interval
Age at exam	1.02	0.67	0.93–1.11
BMI	1.05	0.53	0.85–1.30
Smoke Present vs. past	0.87	0.85	0.22–3.52
25 (OH) vitamin D_3 >30 ng/mL vs. < 30 ng/mL	1.96	0.34	0.49–7.76
Total duration of antiretroviral <1 year <1year/3 years 5 years	 0.30 0.68	 0.19 0.66	 0.05–1.88 0.12–3.87
Nadir CD4 cells/mm³	1.00	0.93	0.99–1.00
Lowest CD4	0.99	0.75	0.93–1.06
Tenofovir treatment	3.79	0.14	0.65–21.9
Tenofovir treatment (years) >3 years vs. <3 years	3.15	0.17	0.61–16.3
Alcohol (yes vs. no)	0.53	0.37	0.13–2.14
Physical activity (yes vs. no)	1.03	0.97	0.26–3.99
Fractures in the past (yes vs. no)	0.94	0.94	0.18–5.02

and P=0.015, respectively) (Table 2).

In a multivariate analysis of the study population, we found that HIV infection and levels of vitamin D < 30 ng/mL were significantly associated with lower T-scores (Table 3-B). However, in the HIV-infected patient group, no correlations were observed between traditional factors associated with lower SI (Table 4).

Discussion

A meta-analysis study published recently[22] found that the odds ratio (OR) of developing osteopenia/osteoporosis in HIV-infected and HAART-treated individuals were approximately two times more when compared to their respective controls. According to the findings of the meta-analysis, they recommend a DXA scan in HIV-infected individuals aged ≥40 years old who have a FRAX score ≥10%, men aged ≥50 years, postmenopausal women, individuals with a history of fragility fracture, individuals receiving chronic glucocorticoid treatment and individuals who are at high risk for falls.

As mentioned previously, DXA is the gold standard reference method for diagnosing osteopenia/osteoporosis. However, it is not always easily available in all the centres.

In our study, we found that the QUS is an available tool for an easy and cheap screen of bone quality. We found significantly lower calcaneus SI in HIV-infected men (P=0.026) compared with age and sex controls from the general population in Israel. A recent study in Dakar, Senegal has also employed ultrasound to determine bone quality and has found it a useful tool for assessing bone health, especially in areas where DXA is unavailable[15].

Bone turnover is the main contributor to both quality and quantity of bone. An imbalance between bone resorption and formation leads to a net loss or gain of bone tissue. High bone turnover results in bone loss and abnormal bone microarchitecture, whereas low bone turnover results in increased bone mass[18]. In our study, we found higher bone turnover markers (P1NP and CTx; P=0.33 and P=0.008, respectively) in HIV-infected people than age and sex controls from the general population in Israel.

Vitamin D is vital to the regulation of calcium and phosphorous metabolism, bone formation and mineralisation. It is documented as one of the key factors associated with bone mass and, therefore, important in the management of osteoporosis. Although the optimum level of serum 25 (OH) D3 (the inactive form of vitamin D) in a healthy population is not uniformly accepted, a concentration of at least 30 ng/mL (75 nmol/L) is recognised as the endpoint (lowest) for skeletal health outcomes by most whereas 20-30 ng/mL (50-75 nmol/L) is considered acceptable and desirable[19]. We found that low levels of vitamin D and HIV infection are associated with significantly lower calcaneus QUS SI T-scores in HIV-infected men.

We did not find any association with traditional factors for low SI in HIV patients such as low BMI, smoking, tenofovir treatment, alcohol abuse or physical activity. Our findings partially agree with previous meta-analysis studies that found a decrease in BMD of HIV patients treated with a protease inhibitor or tenofovir compared to their respective controls, but without statistical significance[22].

Traditional osteoporosis risk factors play an important role in HIV-infected individuals[23]. However, HIV-infected

individuals have additional risk factors such as low fat mass, low lean body mass, lipodystrophy and low CD4 cell counts, which contributes to bone loss. In our study we did not find any statistically significant difference in risk factors between HIV and non-HIV-infected patients.

Our study has several limitations. First, our results should be validated by a DXA scan. However, the finding of lower SI and higher bone turnover markers in the HIV group may reflect increased bone resorption presenting in young HIV-infected men. Second, the number of the study participants is small, and our results must be confirmed with a larger study. Third, it would also be worthwhile to assess the calcaneus bone by QUS in HIV-infected young women.

In conclusion, HIV-infected men that participated in the study had significantly lower calcaneus QUS BMD T-scores (P=0.026) and higher bone turnover markers (P1NP and CTX; P=0.33 and P=0.008, respectively) than age and sex controls from the general population in Israel. The main factors that might be responsible for the increased bone resorption in HIV-infected men were the HIV infection and lower levels of vitamin D. We did not observe any association with traditional factors for low BMD in HIV patients such as low BMI, smoking, tenofovir treatment, alcohol abuse or physical activity. We conclude that QUS may be a useful tool for initial, fast and cheap screening of bone quality for discriminating between those not at risk for fracture and those in need of further evaluation by central DXA in HIV-infected patients. We also conclude that much more clinical investigation are necessary to standardize the QUS method and use it safely as a substitute for DXA screening.

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