OSTEOPOROSIS RISK FACTORS IN HIV POSITIVE WOMEN WITH OSTEOPOROSIS: A RETROSPECTIVE ANALYSIS

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Abstract

Multifactorial risk factors such as HIV/HCV co-infection and antiretroviral therapy (ARV) have been associated with osteoporosis in HIV+ women. We retrospectively analysed which known risk factors were associated with the diagnosis of osteoporosis, according to the WHO definition, in HIV positive women who were followed-up at the AIDS Centre of the University of Palermo, Italy between January 2011 and December 2014.

Twenty-one HIV+ women with osteoporosis (13 HIV+ mono-infected and 8 HIV/HCV co-infected females) who underwent dual-energy X-ray absorptiometry (DXA) and liver stiffness assessment were included in the study.

No significant differences between the HIV and HIV/HCV group were found regarding liver stiffness and lumbar/femoral osteoporosis scores. In a univariate analysis, we observed a positive linear correlation between *LBD score* (Lumbar Bone Density) with *pre-fractures* (p-value = 0.0082), *smoke* (p-value = 0.0008), *alcohol* (p-value < 0.0001) and *ARV exposure score* (p-value = 0.0039), while there were no significant negative linear correlations. In multivariate analysis, *pre-fractures*, *smoke* and *alcohol* were positive predictors of *LBD score*, while *previous antiretroviral therapy* (ARV) (years) score was a negative predictor compared to others. Univariate analysis showed a positive linear correlation between *FDB* (Femoral Bone Density) with *smoke* (p-value = 0.0303) and *alcohol* (p-value = 0.0050), while there were no significant negative linear correlations. In multivariate analysis, *alcohol* was a positive predictor of *FDB score* compared to others, while *ARV score* was a negative predictor compared to others.

This preliminary study suggests that other factors besides ARV score and liver fibrosis may affect the skeletal system in osteoporotic women with HIV infection. Some of these factors, such as alcohol and smoking, are modifiable. Additional research into impact on osteoporosis in HIV women with osteoporosis is required.

Introduction

Clinical data accumulated over the past two decades attest to a significant decline in bone mineral density (BMD) in patients infected by HIV, which does not remit but may actually intensify with anti-retroviral therapy (ART). Several studies show that prevalence of osteoporosis is more than three times higher in HIV-infected patients than in HIV-uninfected controls [1-3].

Recent studies have identified a high prevalence of low BMD and osteoporosis among predominately Caucasian HIV infected women with or without chronic hepatitis [4,5].

Much effort has been made to identify the numerous multifactorial mechanisms that may be involved in skeletal disorders in the HIV setting [6-8,30]. In addition to the traditional osteoporosis risk factors that may be more prevalent in HIV-infected patients, such as vitamin D deficiency, low body weight, lifestyle risk factors [6,9] (alcohol consumption, smoking, corticosteroid use and drug addiction), HIV infection and chronic pro-inflammatory status may play a role in decreasing bone mineral density (BMD). Also aging, hepatitis co-infections and other HIV-related infections, antiretroviral therapy (ARV) [26-29] and serotonine use in patients affected by panic disorder have been associated with skeletal disorders and a greater risk of osteoporosis [6-9,25], and consequent common associated pathologies, such as multiple exostoses and osteochondral lesions of the talus with edema, conditioning quality of life [22-24].

Hepatitis C virus (HCV) infection is a major health problem in the HIV-infected population [10-14]. A recent systematic review and meta-analysis suggests that HIV/HCV co-infection is associated with a greater risk of osteoporosis than HIV mono-infection [13], although many divergences exist regarding osteoporosis prevalence in co-infected patients, with values ranging from 5-45%. Moreover, there is some debate over whether liver disease severity may indeed have a relevant role in increasing the risk of osteoporosis in co-infection [12-14].

In this study we describe the characteristics of both HIV mono-infected and HIV/HCV co-infected women with osteoporosis, and we analyze risk factors for decreased bone mineral density (BMD). We investigate which potential metabolic, lifestyle and/or HIV-related risk factors are associated with decreased BMD, and we report a statistical analysis between the two groups to ascertain whether bone loss is more prevalent in HIV/HCV co-infected patients.

Materials and Methods

Data on patients who were followed up at the AIDS Center of the "Paolo Giaccone" University Hospital in Palermo, Italy, in the day care or outpatient facility between January 2011 and May 2013 were extracted from the hospital's medical record database as previously reported [15].

We gathered clinical documentation on 21 HIV-positive women diagnosed with osteoporosis. Osteoporosis was diagnosed when either femoral neck or lumbar spine DXA T-scores were less than -2.5, as recommended by the World Health Organization (WHO) and the National Osteoporosis Foundation [16,17]. The following data were collected and entered into a specially designed database: (a) demographic and clinical characteristics of the patients: age, gender, geographic origin (namely the patient's geographical origin stated in a document certified by the Italian institutions), lifestyle habits (including educational level, drug abuse history, sexual behavior), body mass index (BMI), comorbidities such as HCV co-infection, osteoporosis biomarker, diabetes and hypertension; (b) current $CD4^+$ T lymphocyte counts (cells/μL) determined by flow cytometry, and HIV RNA viral load (VL) measured in plasma by reverse transcriptase polymerase chain reaction (Roche Amplicor; lower limit of quantitation 20 copies/mL, 1.3 $\log_{10}$) as previously reported [5,6]; and (c) variables related to ART, namely date of ART initiation, total time on ART, antiretroviral (ARV) drugs received and degree of adherence. These data were obtained from medical records and previously published investigations [6,12,36]. All patients except the

treatment-naïve subjects had started ART during previous inpatient stays. None of the migrants enrolled in the study had a general practitioner (GP).

Laboratory methods

CD4+ T-cell count (most recent value and nadir) and plasma HIV-RNA levels of all the HIV patients were assessed as previously reported [6,15]. Serum bone alkaline phosphatase, 25-hydroxyvitamin D, phosphorus and calcium levels were obtained for both HIV-infected cases and un-infected controls. Vitamin D assessment was performed in the sunnier months from April through October, since we know that vitamin D formation is not possible or is inadequate [6] during the less sunny months due to Italy's latitude.

Bone mineral density assessment

For both HIV-infected cases and uninfected controls, BMD was assessed at baseline by DXA, using a QDR Discovery Hologic DXA in the femoral neck and DXA in the lumbar spine by total body DXA [5,6]. For each scan, BMD and T-scores were recorded. T-scores compare BMD with the mean of a healthy young (age 20–30 years) reference population, matched for sex and race, and were expressed as the number of standard deviations above or below the reference mean. Osteoporosis was diagnosed when either femoral neck or lumbar spine DXA T-scores were less than -2.5, as recommended by the World Health Organization (WHO) and the National Osteoporosis Foundation [16,17].

Liver fibrosis assessment

Transient elastography (FibroScan®; EchoSens, Paris, France), which assesses liver fibrosis by measuring liver stiffness, was performed on the HIV/HCV co-infected patients. Details of the technical background and examination procedure have previously been described [18]. Liver cirrhosis was defined as liver stiffness ≥ 12.5 kPa according to the standard definition [10,11].

Statistical analysis

Statistical analysis was performed by Matlab statistical toolbox version 2008 (MathWorks, Natick, MA, USA) for Windows at 32 bit, on a random sample of 21 females with osteoporosis, aged from 37-59 years, with mean 47.62 y.o. and standard deviation 8.32 y.o.

In addition, we considered two subgroups, one with patients who tested positive for HIV only, and the other with people who tested positive for HIV/HCV co-infection.

Data were presented as number and percentage for categorical variables, and continuous data were expressed as mean ± standard deviation (SD), unless otherwise specified. The χ^2 test with Yates correction and Fisher's exact test were performed to evaluate significant differences of proportions or percentages between the two groups. In particular, Fisher's exact test was used when the χ^2 test was not appropriate.

We performed the one-way ANOVA test to evaluate significant differences between mean scores for the HIV and HIV/HCV group.

Finally, univariate and multivariate linear correlation analysis was performed, where the test on Pearson's linear correlation coefficient R was performed with t-Student test, under null hypothesis of Pearson's linear correlation coefficient R = 0.

For this step we considered *LBD score* (Lumbar Bone Density) and *FDB score* (Femoral Bone Density) as dependent variables, and *pre-fractures*, *smoke*, *alcohol* and *previous antiretroviral therapy* (ARV) (years) as independent variables. We did not consider age, BMI and menopause among the independent variables as their impact on osteoporosis is already well noted. In particular, we considered continuous experimental probability distribution for dichotomous variables as follows:

- for *smoke* we assigned 1 to patients who smoked and 0 to non smokers;
- for *alcohol*, we assigned 1 to patients who drank alcohol and 0 to those who didn't;
- for *pre-fractures* we assigned 1 to the presence and 0 to the absence of pre-fractures;
- for *infected*, we assigned 0 to HIV infected patients and 1 to HIV/HCV co-infected patients;
- for *assumption of drugs* we assigned 1 for yes and 0 for no.

We considered all statistical tests with p-value < 0.05 to be significant.

In accordance with international standards, a T-score of TR or TF ≤ -2.5 indicated that the patient had osteoporosis.

Results

We consecutively enrolled 21 HIV-positive women (46.92 y.o. ± 3.97 SD): 13 HIV female patients aged 38-53, mean 46.92 y.o. and standard deviation (SD) 3.97 y.o., and 8 HIV/HCV female patients aged 37-79, mean 48.75 y.o. and standard deviation (SD) 12.42 y.o.. Table 1 shows the characteristics of these women.

Comparative analysis of the two groups did not show any statistical difference between HIV mono- and HIV/HCV co-infected osteoporotic women.

As reported in Table 2, univariate analysis revealed a positive linear correlation between LBD score with *pre-fractures* (p-value = 0.0082), *smoke* (p-value = 0.0008), *alcohol* (p-value < 0.0001) and *previous antiretroviral therapy* (ARV) (years) (p-value = 0.0039), while there were no significant negative linear correlations. Multivariate analysis showed that *pre-fractures*, *smoke* and *alcohol* were positive predictors of LBD, while *previous antiretroviral therapy* (ARV) (years) was a negative predictor compared to others. In others words, the presence of *pre-fractures*, *smoke* or *alcohol* implied a higher LBD score, i.e. the patients' osteoporosis score tended towards zero, and the assumption of ARV drugs indicated a higher likelihood of osteoporosis compared to others.

The results of univariate analysis reported in Table 2 show a positive linear correlation between FBD score with *smoke* (p-value = 0.0303) and *alcohol* (p-value = 0.0050), while there were no significant negative linear correlations. Multivariate analysis revealed *alcohol* to be a positive predictor of FBD compared to others, while *previous ARV* was a negative predictor compared to others. In other words, the presence of *smoke* or *alcohol* implied a higher FBD score, i.e. the patients' osteoporosis score showed a tendency towards zero and the assumption of *ARV*

drugs indicated a higher likelihood of osteoporosis compared to others.

Finally, this preliminary study identified no correlation between (mono/co)-infection and LBD or FBD score.

Univariate analysis showed a positive linear correlation between LBD score with *pre-fractures* (p-value = 0.0082), *smoke* (p-value = 0.0008), *alcohol* (p-value < 0.0001) and *previous antiretroviral therapy* (ARV) (years) (p-value = 0.0039), while there were no significant negative linear correlations. In multivariate analysis *pre-fractures*, *smoke* and *alcohol* were positive predictors of LBD, while *previous antiretroviral therapy* (ARV) (years) was a negative predictor compared to others. In others words, the presence of *pre-fractures*, *smoke* or *alcohol* implied a higher LBD score.

Univariate analysis showed a positive linear correlation between FBD score with *smoke* (p-value = 0.0303) and *alcohol* (p-value = 0.0050), while there were no significant negative linear correlations. In multivariate analysis, *alcohol* was a positive predictor of FBD compared to others, while *previous ARV* was a negative predictor compared to others. In others words, the presence of *smoke* or *alcohol* implied a higher FBD score.

Discussion

The authors selected a group of HIV-infected patients with osteoporosis to identify which factors may influence progression to low bone mineral density, in women in particular.

Although our study involved a small group of women, we found no difference in osteoporosis score for HIV mono-infected women and those co-infected with the hepatitis C virus.

Vincent Lo Re demonstrated that, compared to healthy reference patients, HIV/HCV co-infected women had decreased tibial trabecular volumetric BMD, diminished cortical dimensions, and significant endocortical bone loss [4].

In a previous study, the authors found that extent of liver fibrosis, measured by transient elastography, was an independent predictor of bone loss in HIV/HCV co-infection, confirming what we found in our own study [5].

Valentina Li Vecchi et al. [5] found that when patients were stratified by sex, a negative correlation between BMD and severity of liver fibrosis applied to co-infected women only, suggesting that other factors (i.e. sexual hormones) may affect the skeletal system in HIV/HCV co-infection more than liver fibrosis.

These observations appear to be in line with what we observed and have reported in this paper, where other factors such as previous fracture, alcohol and smoking are significantly prevalent risk factors in women with osteoporosis.

In particular, multivariate analysis showed that pre-fractures, smoke and alcohol were positive predictors of LBD score. It also confirmed that the risk factor *alcohol* is a positive predictor for FDB score compared to others.

The risk factor *previous antiretroviral therapy* (ARV) (years) score was a negative predictor for both LBD score and FDB score compared to others.

In a previous study the authors found that, regarding antiretroviral drugs, the use of protease inhibitors (PI) was a risk factor for osteopenia/osteoporosis.

Due to the small number of subjects enrolled in this study, we did not establish which group of ARV drugs is correlated with osteoporosis score, therefore we maintain that number of years of ARV treatment is not a risk factor for osteoporosis.

Among the various factors shown to affect osteoporosis, it is no surprise to us that our study, like others, has confirmed that previous fracture, alcohol abuse and smoking are strong and relevant factors that affect osteoporosis in women. These factors have also been described for men.

Alcohol use was recently correlated with low bone mineral density in adults with HIV infection. This risk factor is one of the modifiable ones for both HIV disease progression and low BMD [19].

Smoking is another lifestyle risk factor. In vitro studies recently demonstrated that nicotine has a negative effect on osteoclastogenesis [19,20].

Costa-Rodrigues J [20] published a manuscript in which cigarette smoke is associated with pathological weakening of bone tissue, and is considered an important playmaker in conditions such as osteoporosis and periodontal bone loss. In addition, it is also associated with an increased risk of failure in bone regeneration strategies.

This knowledge opens up new horizons for the HIV positive patient, in whom smoking is often a risk factor, especially in HIV positive women [21].

Smoking, therefore, is a candidate risk factor not only for cardiovascular disease or tumours, but also for osteoporosis.

Regarding pre-fractures, complications of pertussis including rib fracture were recently reported in an adult with osteoporosis [31,32,39].

As far as surveillance of vaccination coverage among adult populations is concerned, the authors encourage the identification of adults who do not have a regular provider or insurance of vaccination against acellular pertussis, and against other infectious diseases such as zoonoses, leishmaniasis and tuberculosis [33-35,27,38, 40,41].

In conclusion, our study highlights some risk factors correlated with osteoporosis in women with osteoporosis, such as alcohol and smoking. These risk factors are modifiable, and this confirms how education programmes that promote a different lifestyle could mitigate the impact of some HIV-related comorbidities.

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Table 1. Characteristics of patients with osteoporosis by HIV and HIV/HCV group. In addition, in

the last column, we report a statistical analysis between the HIV and HIV/HCV group

Characteristics	HIV (OP)	HIV/HCV (OP)	Statistical analysis: p-value
% patients	61.90 (13/21)	38.10 (8/21)	0.217 (CY)
Age	46.92 ± 3.97	48.75 ± 12.42	0.624 (A)
Pre-fractures	38.46 (5/13)	37.50 (3/8)	1.00 (F)
Menopause	38.46 (5/13)	37.50 (3/8)	1.00 (F)
Smoke	76.92 (10/13)	62.50 (5/8)	0.631 (F)
Previous antiretroviral therapy (ARV) (years)	61.54 (8/13)	50.00 (4/8)	0.673 (F)
Alcohol	38.46 (5/13)	50.00 (4/8)	0.673 (F)
Nadir CD4+	84.62 (11/13)	37.50 (3/8)	0.056 (F)
LBD	-3.38 ± 0.35	-3.56 ± 0.73	0.453 (A)
FBD	-2.23 ± 0.36	-2.39 ± 0.34	0.326 (A)
Undetectable HIV-RNA	76.92 (10/13)	42.86 (3/8)	0.164 (F)
Genotype			
Type 1	84.62 (11/13)	100.00 (8/8)	0.505 (F)
Type 2	0.00 (0/13)	0.00 (0/8)	1.00 (F)
Type 3	7.69 (1/13)	0.00 (0/8)	1.00 (F)
Type 4	7.69 (1/13)	0.00 (0/8)	1.00 (F)
Liver stiffness (kPa)	14.02 ± 13.47	10.43 ± 2.27	0.468 (A)
BMI	23.10 ± 4.10	21.70 ± 4.77	0.483 (A)
Total	**13**	**8**	

(A) = one-way Anova test; (CY) = Chi-square test with Yates continuity correction; (F) = Fisher's exact test; * = significant

Table 2. Univariate and multivariate linear correlation analysis between *LBD score* (Lumbar Bone Density) and *FDB* score (Femoral Bone Density) as dependent variables and *pre-fractures*, *menopause*, *smoke*, *alcohol, infected* and *drugs* as independent variables.

Dependent variable / independent variable	**Univariate analysis**	**Multivariate analysis**
	R (p-value)	*Multiple linear correlation coefficient:* 0.877
LBD / Pre-fractures	0.561 (0.0082) *	*R_partial*: 0.511 p-value = 0.0362*
LBD / Smoke	0.675 (0.0008)*	*R_partial*: 0.492 p-value = 0.0448 *
LBD / Alcohol	0.792 (<0.0001)*	*R_partial*: 0.670 p-value = 0.0032 *
LBD / Previous antiretroviral therapy (ARV) (years)	0.602 (0.0039) *	*R_partial*: -0.587 p-value = 0.0133 *
LBD / Infected	-0.253 (0.269)	*R_partial*: 0.430 p-value = 0.0851
	R (p-value)	*Multiple linear correlation coefficient:* 0.771
FBD / Pre-fractures	0.169 (0.465)	*R_partial*: 0.214 p-value = 0.409
FBD / Smoke	0.473 (0.0303)*	*R_partial*: 0.472 p-value = 0.056
FBD / Alcohol	0.588 (0.0050) *	*R_partial*: 0.561 p-value = 0.0191 *
FBD / ARV	0.276 (0.226)	*R_partial*: -0.556 p-value = 0.0204 *
FBD / Infected	-0.298 (0.190)	*R_partial*: 0.257 p-value = 0.320

* = significance test; R = Pearson's linear correlation coefficient; *R_partial* = the partial correlation coefficient is the coefficient of correlation of the variable with the dependent variable, adjusted for the effect of the other variables in the model