ORIGINAL ARTICLE

EVALUATION OF BONE MINERAL DENSITY AND CORRELATIONS WITH INFLAMMATION MARKERS IN ROMANIAN HIV-POSITIVE PATIENTS UNDERGOING COMBINED ANTIRETROVIRAL THERAPY

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Abstract

The aim of this study was to evaluate the correlations between inflammation and bone mineral density (BMD) in HIV patients undergoing combined antiretroviral therapy (cART) and the risk for BMD disturbances. We performed a cross-sectional study on HIV positive (HIV+) patients, from "Matei Balș" National Institute of Infectious Disease, between June 2008 - 2010. Blood samples were analysed for tumour necrosis (TNF)-alpha, interleukin (IL)-6, C reactive protein (CRP). All patients had dual-energy X-ray absorptiometry (DXA) scan. We enrolled 56 HIV patients, 59% males, with median age of 28 years. According to the T-score, 20% had low BMD, 16% osteopenia and 4% osteoporosis. We divided the patients into 2 groups according to the sex. We found direct correlations between T-score and body mass index (BMI) in males and between T-score and CRP in females. There were no correlations between T-score and standard antiretroviral therapy (ART) duration or the number of cART regimens. There were no differences in T-score for patients with/without protease inhibitors (PI)/non-nucleoside reverse-transcriptase inhibitors (NNRTI) regimens. There is a low prevalence of decreased BMD in young HIV patients undergoing cART. In males, the main risk factor was low BMI. Many patients had systemic inflammation despite effective cART, but there was no correlation between BMD and inflammation.

Rezumat

Obiectivul acestui studiu a fost evaluarea corelațiilor dintre inflamația sistemică și densitatea minerală osoasă (DMO) și a riscului de scădere al DMO la pacienții HIV pozitivi (HIV+) aflați sub tratament. Am efectuat un studiu transversal cu pacienți HIV+ din Institutul Național de Boli Infecțioase „Prof. Dr. Matei Balș", din iunie 2008 pînă în 2010. Au fost dozate TNFalfa, IL6 și proteina C reactivă (PCR) serice. Pacienților le-a fost efectuată procedura dublei absorbții cu raze X (DXA). Au fost cooptați 56 de pacienți HIV+, 59% bărbați, mediana vârstei fiind de 28 de ani. Conform scorului T, 20% dintre pacienți aveau DMO scăzută: 16% osteopenie și 4% osteoporoză. Pacienții au fost împărțiți în 2 grupuri în funcție de sex. Am stabilit corelații directe între scorul T și indicele de masă corporală (IMC) la bărbați și între scorul T și PCR la femei. Nu s-au înregistrat corelații între scorul T și durata terapiei sau numărul de scheme folosite. Nu au existat diferențe între scorul T al pacienților aflați / nu în tratament cu inhibitori non-nucleozidici de revers-transcriptază (INNRT)/inhibitori de protează (IP). Prevalența valorilor DMO scăzute la pacienții HIV tratați a fost scăzută. În cazul bărbaților, principalul factor de risc a fost IMC-ul scăzut. Mulți pacienți au prezentat inflamație cronică în ciuda terapiei, fără corelație cu DMO.

Keywords: HIV, bone mineral density, systemic inflammation, antiretroviral treatment

Introduction

Nowadays, HIV infection is considered a chronic infection due to the progresses of the combined antiretroviral treatment (cART) which lowered the morbidity and mortality of HIV infected patients. However, an accelerated aging process was observed in these patients, which consists in developing age-related diseases like ischemic heart disease, arterial hypertension, chronic renal disease and osteoporosis at younger ages compared to general population. The researchers are suggesting that this process is caused by the chronic inflammatory status characteristic for HIV positive patients, regardless the effective antiretroviral treatment [2, 7, 16].

Many studies have shown that HIV infection is responsible for bone metabolism disorders. The skeletal system is characterized by permanent remodelling with a perfect balance between bone deposition and removal. After the age of 40, bone loss is accelerated, leading to a slow decline in bone mineral density (BMD) [20]. In HIV infected patients, osteopenia

and osteoporosis are diagnosed at younger ages. The probability to develop osteopenia or osteoporosis can be over 6-fold and almost 4-fold higher in HIV positive patients than in uninfected persons, respectively [12]. Thus HIV infected patients have a 3-fold increased risk of fractures compared with that of age and gender matched HIV negative patients [24].

There are several conditions considered as traditional risk factors for osteopenia/osteoporosis which include older age, female sex, low body mass index (BMI), heavy smoking, alcohol consumption, a sedentary lifestyle, hypogonadism and vitamin D deficiency and some antiretroviral drugs. Several disorders can disturb bone turnover. In case of HIV-positive patients, beside the risk factors already mentioned, there are studies suggesting that cART and the presence of chronic inflammation could increase the risk of a low BMD, however their implication it is still debated [6, 8, 14, 18, 19].

The role of chronic inflammation in bone metabolism disturbances was suggested by the fact that in other disorders associated with chronic inflammation like rheumatoid polyarthritis, systemic lupus and inflammatory bowel diseases, osteopenia and osteoporosis are diagnosed at younger ages then in general population. An explanation for this could be that the osteoclasts have the same origin in the same precursor cells as the monocytes and the factor that activates the osteoclasts is a cytokine that also induces T cell differentiation and controls dendritic cell function [19, 20].

Our objective was to evaluate correlations between systemic inflammation markers and bone mineral density in HIV-infected patients undergoing cART. We also aimed to assess possible associations between cART characteristics (such as treatment duration and number of regimens) and the risk of developing osteopenia/osteoporosis.

Materials and Methods

We performed a cross-sectional study on HIV-positive patients, which took place in a tertiary care hospital, the "Prof. Dr. Matei Balş" National Institute of Infectious Diseases, Bucharest, Romania, from June 2008 until June 2010. The local ethics committee approved the study and from all patients we obtained an informed consent form prior to the enrolment. We enrolled HIV-positive consecutive patients, both male and female, over 18-years old, undergoing stable cART for at least 6 months.

During the study visit the patients completed a questionnaire containing demographic information, personal and family history. A complete physical check-up was performed and weight and height were measured. We used the WHO criteria for defining BMI categories: normal weight for a BMI between 18.5 - 24.99 kg/m^2, overweight and obesity for a BMI $\geq$ 25 kg/m^2 [29]. Blood was collected and analysed for tumour necrosis factor alpha (TNF-alpha) - BioSource EASIA KAP 1751, inteleukin-6 (IL-6) - BioSource EASIA KAP 1261 and high sensitive C reactive protein (hsCRP) - nephelometric assays.

BMD was assessed in all patients by whole body dual energy X-ray absorptiometry (DXA) using Lunar scanner. Usually, BMD is expressed by the T-score and Z-score. T-score compares the subject`s BMD with the average BMD of a young normal adult around 30 years, when the peak bone density is achieved. The BMD is considered normal when the T-score is higher than -1. Osteopenia is considered in case of a T-score between -2.4 and -1 and osteoporosis is diagnosed when the T-score is less than -2.5. Z-score compares the subject`s BMD with the average BMD of people having the same age, race and gender like the investigated subject. A value less than -2 for the Z-score is a diagnostic test for osteoporosis [28].

For statistical analyses we used Statistical Package for the Social Sciences (SPSS) (version 21, USA). In descriptive analysis, we expressed the non-parametrical continuous variables as medians and interquartile ranges (Q1, Q3) and categorical variables as percentages. To test for statistical significance between groups, we used Chi-square for categorical variables and Mann-Whitney U test for non-parametric continuous variables. Based on the observation made in the univariate analysis, we constructed a linear regression model to test the association of BMD expressed as T-score with normally distributed continuous variables. We calculated the coefficient of determination to assess these linear correlations. Statistical significance was considered for a p-value $\leq$ 0.05.

Results and Discussion

We enrolled 56 HIV-positive patients, receiving stable cART for at least 6 months, consecutively as they came for the monthly routine check-up. The patients were predominantly male, with a median age of 28 (20 - 38.5) years and the mode of 20 years, 76% of patients with undetectable HIV viral load. The whole group of patients had a median value for T-score of +0.1 (-0.9 - +0.67) and for Z-score of +0.15 (-0.57 - +0.7). According to the T-score 20% of the patients had an abnormal BMD, 9 patients having osteopenia (16%) and 2 patients having osteoporosis (4%). According to the Z-score, only one patient had severe bone demineralization. The patients were divided into Group 1 (male patients) and Group 2 (female patients), to avoid any interference of hormonal factors into the statistical analysis. The demographic data and

studied parameters for the 2 groups are presented in Table I.

When we analysed at the inflammation markers, we observed pathological values for TNF-alpha, IL-6 and hsCRP in 59%, 4.5% and 37.5% of male patients respectively and 53%, 10.5% and 38.5% of female patients respectively. Regarding the anti-retroviral drug classes, 75.8% and 39.4% of male patients and 78.3% and 43.5% of female patients received treatment combinations containing protease inhibitors (PIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs) respectively.

Table I

Demographic characteristics, bone mineral density and inflammation markers of male compared to female patients

Characteristic	Group 1	Group 2	p-value[3]
Number	33	23	
Age[1], years	31 (20.5 , 39)	22 (20 , 37)	0.262
BMI[1], kg/m^2	24.2 (22 , 27.4)	20.5 (19.1 , 23.9)	0.001
Smoking[2]	34.5/10	18.8/3	0.322
Time on cART[1], months	44 (22 , 110)	50 (26 , 103.5)	0.366
Total number of ARV combinations[1]	1 (1 , 3)	2 (1 ,3)	0.644
T-score[1]	+0.1 (-1.25 , +0.8)	+0.1 (-0.7 , +0.4)	0.764
Z-score[1]	0 (-0.9 , +0.6)	+0.4 (-0.4 , +0.9)	0.286
Osteopenia according to T-score[2]	25/7	8.7/2	0.145
Osteoporosis according to T-score[2]	7/2	0/0	0.488
TNF-alpha[1], pg/mL	14.8 (6.5 , 43.6)	13.2 (2.7 , 41.1)	0.464
IL-6[1], pg/mL	11.1 (7.6 ,17.5)	7.6 (5.4 , 10.8)	0.075
CRP[1], mg/L	2 (0.9 , 7.2)	1 (0.5 , 3.7)	0.288

p-values were calculated with Mann-Whitney U test; BMI = body mass index; TNF = tumour necrosis factor; IL-6 = interleukin-6; CRP = C-reactive protein. [1]Median (interquartiles range); [2]Percent/number; [3]p-values for Group 1 *vs.* Group 2

We investigated into the 2 groups the presence of significant statistical correlations between T-score on one hand and BMI, inflammation markers and relevant information regarding ART treatment (total duration on cART, total number of antiretroviral combinations) on the other hand. We found a directly proportional correlation between T-score and BMI (Spearman`s rank correlation coefficient (rho) = 0.484, p-value = 0.004), for Group 1 (Table II) and between T-score and hsCRP (Spearman`s rho = 0.691, p-value = 0.009), for Group 2 (Table III).

Table II

The correlation of T-score with different variables, for Group 1

	T-score	
	Spearman`s rho	**p-value**
Age	0.209	0.244
BMI	**0.484**	**0.004**
TNF-alpha	0.272	0.222
IL-6	0.149	0.509
hsCRP	0.326	0.217
Time on cART	0.246	0.223
Total number of ARV combinations	0.073	0.685

We found no significant correlations between T-score and duration of cART, neither in the male group, nor in the female group of patients. Also for both groups, there was no correlation between T-score and the total number of antiretroviral combinations that the patients were receiving since the infection diagnosis and the enrolment into the present study. Regarding the antiretroviral classes, we found no difference in T-score for patients either with or without PI containing regimens (p-value > 0.005), or with or without NNRTI containing regimens (p-value > 0.005).

Table III

The correlation of T-score with different variables, for Group 2

	T-score	
	Spearman`s rho	**p-value**
Age	0.004	0.987
BMI	0.076	0.731
TNF-alpha	-0.249	0.305
IL-6	-0.271	0.261
hsCRP	**0.691**	**0.009**
Time on cART	0.072	0.790
Total number of ARV combinations	-0.120	0.585

We constructed further a step-wise forward model of regression and we found for Group 1 significant values for R squared coefficient (R^2 = 0.214) and for the coefficient of determination R (R = 0.463) defining the correlation between T-score and BMI (Table IV). In a same step-wise forward model of regression for Group 2 we found a strong correlation between T-score and hsCRP, is a strong one as defined by the values of R squared coefficient (R^2 = 0.309) and coefficient of determination R (R = 0.556) (Table V).

Table IV

Linear regression model with T-score as dependent variable and BMI as independent variable, for Group 1

	B	β	Confidence interval	R	R^2	p-value
BMI	0.189	0.463	0.056 - 0.322	0.463	0.214	0.007

Table V

Linear regression model with T-score as dependent variable and hsCRP as independent variable, for Group 2

	B	β	Confidence interval	R	R^2	p-value
hsCRP	0.162	0.556	0.001 - 0.323	0.556	0.309	0.048

However, after we adjusted the two regression model for age, none of the two correlations (between T-score and BMI for male patients and between T-score and hsCRP for female patients) remained statistical significant.

Our study showed that 20% of HIV-treatment multi-experienced patients had a low BMD according to the T-score, 16% and 4% of patients had osteopenia and osteoporosis respectively. When we divided the study population in 2 groups according the sex, we observed that T-score was significantly correlated with BMI for male patients and with hsCRP for female patients; however these correlations did not remain significant when we adjusted the models for age.

Our study population is representative for HIV-positive population in Romania, which has distinctive epidemiological features from those in Western European countries: a young population infected during the childhood, with a subtype F viral strain in the late `80s, with an equal sex distribution, the majority of them being slow progressors and multi-experienced to cART [3, 4, 22]. To our knowledge, this is the first study investigating the prevalence of low BMD in Romanian HIV-positive population. Our results showed a low prevalence of osteopenia/ osteoporosis among people living with HIV, compared to other studies that have reported rates between 39 to 54% for osteopenia and between 12 to 24% for osteoporosis. Apart from the other studies that investigated the BMD changes predominantly in male patients over 40 years old, our study participants had an almost equal sex distribution and were significantly younger [5, 14, 20, 25]. Our particular finding – low BMD in 20% of young HIV-positive patients – is concerning, because around the age of 30 changes in BMD are not usually expected, at this age the BMD being considered to reach a maximum [18, 20]. But in this population changes in BMD are a reality and clinicians should be aware of them because of the increased risk of fractures [18]. The international recommendations are that in men under 50 years and in premenopausal woman BMD should be assessed using Z-score [20, 28]. However the major limitation of this score is its low sensitivity - around 50%, which is why the majority of the studies are using only T-score or both scores together for estimating BMD [1].

Regarding the risk factors for low BMD, many studies pointed out that a low BMI predisposes to osteopenia and/or osteoporosis, not only in general population, but also in HIV-positive patients [5]. A recent study carried on 918 HIV-positive patients, predominantly males, showed that a low BMI is an important risk factor for a decreased BMD and stressed out the importance of DXA evaluation especially in underweight patients [23]. Brown *et al.* investigated the association between BMD and lean and fat body mass in HIV naive patients and they found that BMD was strong correlated with the lean body mass and moderate correlated with the total fat mass. Regarding the lean mass, it was considered an important predictor for fracture occurrence in general population, but the implication of the total fat mass in changes of BMD was not been totally clarified [6]. The results of our study showed likewise the correlation between low BMI and the presence of osteopenia/osteoporosis; however, this association was present only in the male group and not in the female group of patients, probably due to the small sample size.

Systemic inflammation and its consequences is a subject much debated in the presence of HIV infection. The hallmark of HIV infection is the chronic immune activation and a persistent inflammatory state, due to the microbial translocation through the intestinal wall. Furthermore this inflammatory state persists even in patients undergoing effective cART, this being supported by the presence in blood of increased levels of inflammation cytokines – TNF-alpha, IL-6, hs-CRP [6, 14, 19]. From the patho-physiological point of view, it is considered that HIV viral proteins, as well as inflammation cytokines TNF-alpha and IL-6 are stimulating the activity of osteoclasts, while osteoblasts activity is suppressed [14]. However, the studies are showing conflicting data regarding the link between systemic inflammation and changes in BMD. A study on 47 HIV naive patients found that increased IL-6 levels were correlated with the progression of the existing osteopenia or osteoporosis, while TNF-alpha levels were not correlated with BMD [14]. Another study on 331 HIV naive patients did not observe any association between BMD and IL-6 and hsCRP levels and concluded that further studies to investigate this relationship are needed [6]. The data regarding the levels of hsCRP and changes in BMD are conflicting not only in HIV population, but also in general population. There are studies suggesting that high levels of hsCRP are associated with a low BMD, while others studies did not found any association between these two parameters; however the levels of hsCRP may represent a risk factor for fractures

occurrence, independent of the BMD value [9, 10, 15]. In our study, we observed increased values of TNF-alpha and hsCRP in more than half and approximately one third of the patients respectively, despite effective antiretroviral treatment. We did not found any correlation between BMD and TNF-alpha and IL-6 neither in men, nor in the female group of patients. We found an unexpected association between BMD and hsCRP in the female group, in the way that elevated hsCRP appeared to be protective for the loss of BMD. Nevertheless, when we adjusted for age in this group, the correlation did not remain significant. One explanation for the unpredicted results could be the small size of the investigated group.

Regarding the role of ART in bone metabolism disturbance, available data is conflicting. Despite beneficial effects on other HIV associated disorders, ART seems to have a negative influence on bone mineralization. Studies have demonstrated that the patients undergoing ART have a 2.5-fold higher risk for osteopenia compared with naive patients [24]. Also, osteoporosis associated fractures are more frequent in patients with continuous ART compared with those with intermittent ART [11]. ART associated bone loss appears early after initiation, but it stabilizes after about 2 years of treatment [13]. The results of our study did not show any association between low BMD and treatment duration or total number of antiretroviral regimens, probably due to the small size of studied groups.

Regarding the different antiretroviral drugs, tenofovir and protease inhibitors are the most common molecules associated with bone loss [21]. The trials which compared BMD in tenofovir *versus* non-tenofovir treatment schemes showed that tenofovir is responsible for an important decrease of BMD in the hip and spine [17, 26]. Tenofovir treatment is associated with low values of 1,25(OH) vitamin D. In this case, renal toxicity with proximal tubule impairment is the most incriminated mechanism which leads to a lower rate of renal hydroxylation of 25(OH) vitamin D [13]. Another factor responsible for bone metabolism disorders is the high concentration of PTH also observed in patients with low vitamin D levels undergoing tenofovir treatment [13]. Tenofovir became available in Romania only in the last few years and no patient from our study was on tenofovir containing regimen.

Randomised studies which compared BMD in patients under Protease inhibitor (PI) *versus* non-PI regimens had conflicting results, but cumulative exposure to boosted PI seems to be associated with a high risk of fragile fractures (patients under treatment with lopinavir/ritonavir have a 17% higher risk of hip, vertebral or wrist fracture) [27]. There are many mechanisms through which PI treatment leads to bone loss: PI may be responsible for an increased resorbtion of osteoclast and inhibition of osteoblast rebuilding function. Some studies showed that PI may cause phosphate balance impairment and/or changes in vitamin D and PTH metabolism [13]. The majority of both male and female patients from our study received at least one antiretroviral combination containing a PI. The most used PI in both groups was boosted lopinavir; nevertheless the presence of PI containing regimens was not associated with a low BMD.

Efavirenz is also associated with BMD disturbances. Low values of vitamin D may be responsible for this effect, but the mechanism which leads to vitamin D metabolism impairment is still unclear [13]. Immune reconstruction achieved under ART may also play a role in BMD loss. Changes in the concentrations of circulating cytokines may provide an explanation for this [13]. In our study, the presence of efavirenz containing regimens was not associated with low BMD; a possible explanation could be the relative small number of patients enrolled into the study.

One of the study limitation is represented by the heterogeneous therapeutically regimen with a wide range of combinations (from 1 to 10 regimens, 21% of patients received more than 3 antiretroviral combination and at least 2 different PIs). We consider that another limitation was the evaluation of BMD through whole body DXA examination, instead of lumbar spine or hip examination.

Conclusions

We report a low prevalence of osteopenia and osteoporosis in a young cohort of HIV positive patients undergoing stable antiretroviral therapy compared to the literature data. For the male patients the main risk factor for low BMD was a low BMI. Despite the low prevalence, the results are important stressing out the role of DXA evaluation as part of the routine check-up even in young people, especially with a low BMI. We found that an important proportion of patients had systemic inflammation despite effective antiretroviral therapy; however there was no significant correlation between BMD and inflammation markers.

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Competing interests

The authors declare that they have no competing interests.

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