BONE QUALITY IN A YOUNG COHORT OF HIV-POSITIVE PATIENTS

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Abstract

Context. In HIV+ patients, several factors related to patient and antiretroviral therapy (ART) could determine early onset of bone mineral density (BMD) disturbances.

Objective. Evaluation of bone quality according to gender in patients from the HIV Romanian cohort.

Design. A cross-sectional study in “Prof. Dr. Matei Balș” National Institute for Infectious Diseases, Bucharest between 2016-2018.

Subject and Methods. We collected data regarding HIV infection, ART history, viral hepatitis co-infections and we calculated patients body mass index (BMI). CD4 cell count, HIV viral load (VL), vitamin-D levels were determined. Dual-energy X-ray absorptiometry (DXA) scans were used to evaluate BMD.

Results. We enrolled 97 patients with the median age of 26 years. According to the DXA T-scores, 10 males and 8 females had osteopenia and 4 males and 4 females had osteoporosis. According to Z-scores 2 males and 1 female had osteoporosis. Hip DXA T-scores revealed osteopenia in 6 males and 9 females, whereas T and Z-scores showed osteoporosis in 2 males and 3 females. Lumbar spine (LS) T-score diagnosed osteopenia in 9 males and 6 females, while T and Z-scores revealed osteoporosis in 3 males and females. In males, low T-scores were associated with decreased BMI; low LS DXA Z-scores with low vitamin-D levels; low T and Z-scores and LS-BMD with high VL.

Conclusions. Evaluating bone quality in patients with a long history of HIV infection, multiple factors should be taken into account.

Key words: HIV, osteopenia, osteoporosis, gender.

INTRODUCTION

Osteoporosis is a disorder characterized by the imbalance between the process of bone production and bone resorption and is associated with decreased bone mineral density and enhanced bone fragility. Osteoporosis is often associated in the elderly

population with fragility fractures such as femoral neck, vertebral and distal radial fractures which generate high mortality and morbidity among these patients. In the European Union, it was estimated that one in three females and one in five men will develop a fragility fracture during their lifetime (1). Bone loss is a multifactorial disorder involving risk factors: female sex, age above 65 years old, smoking, low body mass index (BMI), low serum levels of vitamin D, endocrine disorders (hypogonadism, hyperthyroidism, Cushing syndrome, hyperparathyroidism, diabetes mellitus), hematologic diseases (multiple myeloma, thalassemia), gastrointestinal diseases (malabsorption, celiac disease), rheumatologic diseases (rheumatoid arthritis, lupus erythematosus, scleroderma), renal diseases, use of certain drugs (glucocorticoids, diuretics) (2, 3).

In many studies, HIV infection was also associated with low bone mineral density and osteoporosis, HIV infected patients having a higher risk to develop osteoporosis and osteopenia compared to the general population (4–7). Since the life expectancy of patients living with HIV infection increased after combined antiretroviral therapy became available worldwide, age-related disorders became more frequent in this category of the population (8). The factors involved in bone tissue loss in the HIV positive population are not entirely known. Antiretroviral therapy (ART), presence of chronic inflammation and some viral proteins seem to be responsible for bone metabolism disturbances (5, 9, 10).

In 2018 more than 15000 HIV infected patients were living in Romania. Most of them (8122 patients) were between 25 and 34 years old. An important part of these young patients is part of the Romanian cohort (11) which consists of seropositive patients who had HIV parenteral exposure during their childhood, in the late '80s. The particularity of this cohort is that have a long history of HIV infection and they were exposed to

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most of the antiretroviral (ARV) drugs for a long time.

The presence of osteoporosis in young and active patients can lead to complications such as fractures that may have an important impact on the patients' quality of life. In these circumstances, it is very important to be aware of the different risk factors that are affecting the young HIV infected patients that subsequently lead to the decrease of bone mineral density.

Dual-energy X-ray absorptiometry (DXA) is widely used for the measurement of bone mineral density. Many scores have been developed for the assessment of the risk for osteoporotic fractures, some of them incorporate DXA scan results (Trabecular Bone Score, FRAX score) while others are based on clinical signs and symptoms (Osteoporosis Risk Assessment Instrument - ABONE, Osteoporosis Self-assessment Tool equation - OST, OSTEORISKAPP). However, all of them have limitations in patients with certain associated disorders and they cannot be generally used for young patients (12, 13).

We aimed to perform a comparative clinical and epidemiological assessment of osteoporosis and osteopenia and associated risk factors between men and women undergoing ART, included in the HIV Romanian cohort.

PATIENTS AND METHODS

We performed a cross-sectional study including HIV positive patients from the Romanian cohort that were followed in "Prof. Dr. Matei Balș" National Institute for Infectious Diseases (MBNIID), Bucharest. The study was approved by the ethics committee and it took place in MBNIID, one of the largest infectious diseases medical centers from Eastern-Europe, between 2016 and 2018. All patients signed the informed consent to participate in this study. The study enrolled HIV positive patients from the Romanian cohort (patients parenterally infected with HIV during their childhood) who agreed and were able to sign informed consent. Pregnant women and patients under 18 years old were excluded.

Data regarding HIV infection, time of diagnosis, viral co-infections (hepatitis B virus (HBV) and hepatitis C virus (HCV)) and ART history were obtained from patients' medical files.

We also performed a complete physical check-up, recording patients' weight and height and we calculated patients' body mass index (BMI). We considered normal body weight if BMI had values

between 18.5 and 24.99 kg/m². Patients with BMI under 18.5 kg/m² were considered underweight and patients with a BMI of over 25 kg/m² were considered overweight.

Blood samples were collected to determine CD4 cell count, HIV viral load, 25-OH- vitamin D. CD4 cell count was performed using the BD FACSCanto® II system. Cobas® TaqMan® 48 system (Roche Diagnostics, Basel, Switzerland) with a detection limit of 20 copies/mL was used to determine HIV viral load and 25-OH-vitamin D levels were determined through electrochemiluminescence immunoassay method (ECLIA). Vitamin D levels over 30ng/mL were considered optimal, between 10-30ng/mL were considered suboptimal and under 10ng/mL – vitamin D deficit.

Bone mineral density (BMD) was evaluated using dual-energy X-ray absorptiometry (DXA) scans. We used the GE Lunar DPX-NT (General Electric Company, New York, Connecticut, USA) scanner available in the MBNIID radiology department.

T-score obtained from the DXA examination is a comparison between patients' BMD and the BMD of a healthy, young adult (reference healthy population aged 20-29 years). Z-score represents a value obtained by comparing the patients' BMD with the BMD of a patient of the same age.

Normal BMD was considered for T scores higher than -1SD. We considered osteopenia for T scores between -2.49SD and -1SD and osteoporosis for T scores under -2.5SD. The World Health Organisation (WHO) recommends that in female patients before menopause and in male patients younger than 50 years old, BMD to be evaluated using DXA Z-scores. Z-scores higher than -2SD were considered within the expected range and Z-scores below -2SD were considered with a risk for secondary osteoporosis.

For statistical analysis, we used software R, version 3.4.4 (The R Foundation for Statistical Computing - Vienna, Austria). We used visual inspection of histograms and the Shapiro-Wilk test to assess if the continuous variables were normally or non-normally distributed. Normally distributed variables were expressed as mean $\pm$ standard deviation (SD) and non-normally distributed variables were expressed as the median and interquartile range (IQR). Categorical variables were expressed as percentages. Pearson product-moment correlation coefficient (PCC) was used to compute linear correlations between normally distributed continuous variables and Spearman's rank-order correlation (rho) to establish correlations

between non-normal distributed variables. Statistical significance was considered for p -values ≤ 0.05 .

RESULTS

We enrolled 97 patients divided into two groups: a male group with 45 patients (46.4%) with a mean age of 25.4 years (range 22- 30) and a female group with 52 patients (53.6%) with a mean age of 25.5 years (range 20-34). Thirty-four patients (35.1%), 20 men (44.4%) and 14 women (26.9%) were active smokers at the time of the enrollment. Details regarding age, viral hepatitis co-infections, nutritional status, and HIV infection are presented in Table 1.

Serum 25-OH-vitamin D levels were determined in 62 patients, in the other 35 patients, this investigation was not done. The mean 25-OH-vitamin D level was 21.7 ± 11.2 ng/mL. Abnormal levels were obtained in 48 patients (77.4%), 21 (67.8%) males and 27 (87.1%) females. There were no significant

differences between vitamin D levels in the male vs. female group. Data regarding 25-OH-vitamin D status and details for each group are listed in Table 2.

Bone mineral density (BMD) was evaluated in all patients through DXA.

According to T score 18 patients (18.6%) were diagnosed with osteopenia (10 males and 8 females), and osteoporosis was diagnosed in 4 males and 4 females. Three patients (3.3%) (2 males and 1 female) had a Z score < -2 SD.

According to T scores obtained at hip examination 6 (19.4%) males and 9 (23.1%) females had osteopenia and 2 (6.5%) males and 3 (7.7%) females had osteoporosis. A total of 5 patients had a Z score below the expected range (2 (6.5%) males and 3 (7.7%) females) – Table 3.

Fifteen patients (21.4%) were diagnosed with lumbar spine osteopenia according to the T scores, most of them males (9 patients). Osteoporosis was diagnosed in 6 patients (8.6%) (3 males and 3 females), according

Table 1. Epidemiological characteristics and HIV immunovirological status of the study patients by gender

	All patients (n=97)	Male group (n=45)	Female group (n=52)	p-value
Median age ¹ (years)	26 (24-27)	26 (25-27)	25 (24-27)	0.97
Current smoker, no. of patients ²	34 (35.1%)	20 (44.4%)	14 (26.9%)	0.071
Co-infections ²				
HBV	30 (32%)	10 (22.2%)	21 (40.4%)	0.056
HCV	3 (3.2%)	2 (4.4%)	1 (1.9%)	0.47
HBV + HDV	2 (2.1%)	2 (4.5%)	0	0.12
Median BMI ¹ (kg/m ²)	21.3 (18.5-23.5)	22.8 (19.5-24)	20.4 (18-22.4)	0.015*
Nutritional status ² ,				
Underweight	23 (23.7%)	9 (20%)	14 (26.9%)	0.42
Overweight	12 (12.4%)	7 (15.6%)	5 (9.6%)	0.34
Obese	2 (2.1%)	2 (4.4%)	0	0.12
Mean time from HIV diagnosis ³ , years	16.7 ± 4.54	17.9 ± 3.94	15.8 ± 4.82	0.024*
Median ART schemes ¹	5 (3-6)	5 (2-7)	4.5 (3-6)	0.74
Median time of exposure to ART ¹ , years	15 (12-17)	15 (13-17)	14 (11.8-16.3)	0.34
PI treatment ²	87 (89.7%)	42 (93.3%)	45 (86.5%)	0.27
Median PI exposure time ¹ , years	9 (5-13)	10 (8-14)	7.5 (8-13)	0.06
EFV treatment, no. of patients ²	57 (58.8%)	26 (57.8%)	31 (59.6%)	0.85
Median EFV exposure time ¹ , years	2 (0-6)	2 (0-5)	1 (0-7)	0.66
TDF treatment ²	13 (13.4%)	3 (6.7%)	10 (19.2%)	0.07
HIV plasma viral load categories ² ,				
Undetectable	60 (61.9%)	28 (62.2%)	32 (61.5%)	0.94
< 200	17 (17.5%)	6 (13.3%)	11 (21.2%)	0.31
> 200	20 (22.7%)	11 (24.4%)	9 (17.3%)	0.38
Median CD4 level ¹ , cells/mm ³	365 (299-746)	552 (246-801)	513 (319-696)	0.87
CD4 cell categories ² ,				
> 500	53 (54.6%)	26 / 57.8	27 / 51.9	0.564
200 - 499	33 (34%)	11 (24.4%)	23 (44.2%)	0.042*
< 200	10 (10.3%)	8 (17.8%)	2 (3.8%)	0.024*

¹Median (interquartile range); ²Number/percent; ³Mean $\pm$ standard deviation; * $p \leq 0.05$, BMI – body mass index, ART – antiretroviral treatment, PI – protease inhibitors, EFV – Efavirenz, TDF tenofovir disoproxil fumarate.

Table 2. Serum vitamin D levels in the enrolled patients

	All patients (n=62)	Male group (n=31)	Female group (n=31)	p-value
25-OH-vitamin D ¹ , ng/mL	21.70 ± 11.2	21.3 ± 11.4	19.8 ± 10.8	0.16
25-OH-vitamin D levels category ²				
suboptimal	39 (62.9%)	18 (58.1%)	21 (67.7%)	0.43
deficit	9 (14.5%)	3 (9.7%)	6 (19.4%)	0.27

¹Mean $\pm$ standard deviation; ²Number/percent.

to both T and Z scores – Table 4.

In the same group, low vitamin D levels were associated with a low Z score ($\rho=0.478$, $p=0.025$) at lumbar spine DXA. High HIV viral loads were associated with low BMD ($\rho=-0.408$, $p=0.007$), T ($\rho=-0.425$, $p=0.005$) and Z ($\rho=-0.414$, $p=0.011$) scores values and low BMD ($\rho=-0.338$, $p=0.047$) at lumbar spine examination. We found no significant correlations between DXA results and the time from the HIV infection diagnosis, current CD4 cell count or ART exposure time. The time of exposure to IP, EFV, and TDF did not influence the DXA results of the examined sites.

In the female group, low BMI was associated with low T score ($\rho=0.289$, $p=0.049$), while BMD was correlated with long exposure to PI ($\rho=-0.302$, $p=0.037$). The other ARV drugs (EFV and TDF) were not associated with DXA results. In this category of patients, we found no correlations between DXA results and 25-OH-vitamin D levels or viral load levels.

DISCUSSION

This paper presents the results of one of the few studies that focus on the bone quality of the young HIV positive population. This kind of patient is representative for the HIV positive population from Romania, where over 10000 children were parenterally infected with HIV in the late '80s (11).

Although in young patients WHO recommends BMD to be evaluated using DXA Z-scores, in this study we took into account both T and Z scores because only a few patients had abnormal Z score, thus the results

obtained using only Z score would not be significant.

The results of this study show a relatively high prevalence of osteopenia (15% at both hip and lumbar spine examination) and osteoporosis (5% according to both T and Z scores obtained at the lumbar spine and hip examination), taking into consideration the young age of the patients enrolled. The hip region was more often affected by osteopenia and osteoporosis in the female group, while in the male group osteopenia was more often diagnosed in the lumbar spine area. These findings are in concordance with another study from Romania (14).

Low BMI and 25-OH-vitamin D serum levels are well-known risk factors for bone mineral density disturbances in both HIV positive and negative patients (15–18). In our study, in the male group, low BMI was associated with low BMD and low T-score values. In another study from Romania that enrolled HIV infected males, low BMD was also associated with low BMI (19). In the female group, we did not find any correlations between BMI and DXA results. Patients with HIV infection are often diagnosed with vitamin D deficiency that can lead to hyperparathyroidism and increased bone mineral density loss (20, 21). In association with classic risk factors responsible for this abnormality, like low sunlight exposure or inappropriate vitamin D diet intake, in HIV infected patients antiretroviral treatment and chronic inflammation may have an important role in vitamin D metabolism disturbances (22). In our study out of 62 patients, 48 had low 25-OH-vitamin D values, 21 of them being male patients and 27 female patients. These percentages are similar to those observed in

Table 3. Hip DXA results of the studied patients, by gender

	All patients (n=70)	Male group (n=31)	Female group (n=39)	p-value
BMD ¹ , g/cm ²	1.01 (0.89-1.10)	1.09 (0.96-1.12)	0.92 (0.81-1.04)	0.004
T score ²	-0.269 ± 1.27	-0.21 ± 1.33	-0.30 ± 1.23	0.77
Z score ²	-0.065 ± 1.27	-0.09 ± 1.24	-0.04 ± 1.32	0.87
T score osteopenia ³	15 (21.4%)	6 (19.4%)	9 (23.1%)	0.70
T score osteoporosis ³	5 (7.1%)	2 (6.5%)	3 (7.7%)	0.84
Z score <2 SD ³	5 (7.1%)	2 (6.5%)	3 (7.7%)	0.84

¹Median (interquartile range); ²Mean ± standard deviation; ³Number/percent; BMD – bone mineral density

Table 4. Lumbar spine DXA results of the enrolled patients, by gender

	All patients (n=70)	Male group (n=31)	Female group (n=39)	p-value
BMD ¹ , g/cm ²	1.12 ± 0.13	1.12 ± 0.13	1.12 ± 0.13	0.98
T score ¹	-0.60 ± 1.16	-0.76 ± 1.18	-0.48 ± 1.14	0.21
Z score ¹	-0.27 ± 1.15	-0.49 ± 1.21	-0.1 ± 1.08	0.15
T score osteopenia ²	15 (21.4%)	9 (29%)	6 (15.4%)	0.16
T score osteoporosis ²	6 (8.6%)	3 (9.7%)	3 (7.7%)	0.76
Z score below the expected range ²	6 (8.6%)	3 (9.7%)	3 (7.7%)	0.76

¹Mean ± standard deviation; ²Number/percent; BMD – bone mineral density

other studies from different regions of Europe (23–25). In a study from Italy, low serum concentrations of 25-OH-vitamin D were correlated with low BMD in the hip region, while in another study, from all 16 patients diagnosed with vertebral fractures 87% had 25-OH-vitamin D deficiency (26,27). In our study, the results of both T and Z scores obtained at lumbar spine DXA were correlated with 25-OH- vitamin D serum levels in male patients. In the female patient's group, we found no association between 25-OH-vitamin D and DXA results.

Most of the patients (60.9%) had undetectable HIV viral load at the moment of DXA examination. In the male group, in patients with detectable viral load, this parameter was negatively correlated with DXA results and lumbar spine BMD. Because systemic persistent inflammation can impact bone density, an explanation for this fact could be that in patients with high HIV viral loads inflammatory markers may also have higher serum concentrations (28). Also, some recent studies proposed that osteoclasts may be target cells for HIV and enhance their bone resorption activity (29). Other HIV parameters like CD4 cell count, time from HIV diagnosis, time from ARV treatment initiation, were not correlated with any DXA result in our study.

It is well known that some antiretroviral drugs can interfere with bone metabolism. Patients under ARV treatment have three times higher rates for osteoporosis (30). The bone resorption is accelerated in the first 2 years after ART initiation, 2-6% of BMD being lost in this period (31). Use of nucleoside reverse transcriptase inhibitors (NRTI) was associated in many studies with bone tissue loss. Among this category of drugs, tenofovir (TDF) was shown to be responsible for low BMD. The mechanism through which TDF lowers BMD is not well understood, phosphates urinary loss and lactic acidemia that leads to calcium hydroxyapatite loss being some of the hypotheses (32, 33). TDF was introduced in Romania in 2014, therefore it was not correlated with DXA results. Only 13 patients were under treatment with TDF at enrollment and they were exposed for a short period to this drug before the DXA examination. Almost all patients (89.7% of the patients) from our study had protease inhibitors (PI) in their ARV regimens with a median exposure time of 9 (5-13) years. In the female group, long exposure to PI was associated with low BMD at DXA. In many studies, molecules from this class of ARV drugs proved to induce bone loss, most probably due to proliferation and activation of osteoclasts (30, 34, 35). We also

evaluated the impact of Efavirenz, a non-nucleoside reverse transcriptase inhibitor frequently involved in BMD loss, on the bone tissue. Although most patients (57 patients) had this molecule in their ARV schemes, we found no correlation between Efavirenz and BMD. The mechanism through which Efavirenz interferes with bone density seems to be related to vitamin D metabolism (36).

This study enrolled only young HIV infected patients with a long and complex history of HIV infection and ARV treatment. Thus we eliminated some other factors that can cause bone mineral density disturbances, like hormonal disturbances induced by ageing, but despite that, there were important differences between male and female patients. In the male group, low BMD was often diagnosed in the lumbar spine. In the same group, low vitamin D levels were associated with low lumbar spine Z-score, and high HIV viral loads negatively influenced the DXA results. In the female group long treatment with PI was associated with low BMD.

This study had several limitations, such as the low number of enrolled patients or the paucity of information regarding other risk factors for bone metabolism disorders like a history of corticosteroid use or the status of renal function. Also, this is a cross-sectional study, thus the causal effect of the associated factors cannot be evaluated.

In conclusion, we report the prevalence of low bone mineral density in a cohort of young patients with HIV infection. In the female group, hip osteopenia was more frequently diagnosed while in the male group the lumbar spine was the most affected region. In the male group, DXA results were influenced by BMI, vitamin D serum levels and HIV viral load, while in the female group PI exposure time was correlated with low BMD at DXA examination. These factors must be taken into account when evaluating the bone metabolism of patients with a long history of HIV infection.

Conflict of interest

The authors declare that they have no conflict of interest.

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References

1. Svedbom A, Hernlund E, Ivergård M, Compston J, Cooper C, Stenmark J, McCloskey EV, Jonsson B, Kanis JA, EU Review Panel of IOF. Osteoporosis in the European Union: a compendium of country-specific reports. *Arch Osteoporos*. 2013;8:137.
2. Rosen CJ. The Epidemiology and Pathogenesis of Osteoporosis. In: De Groot LJ, Chrousos G, Dungan K, Feingold KR, Grossman A, Hershman JM, *et al.*, editors. *Endotext* [Internet]. South Dartmouth (MA): MDText.com, Inc.; 2000 [cited 2018 Dec 25]. Available from: <http://www.ncbi.nlm.nih.gov/books/NBK279134/>
3. Tarantino U, Iolascon G, Cianferotti L, Masi L, Marcucci G, Giusti F, Marini F, Parri S, Feola M, Rao C, Piccirilli E, Zanetti EB, Cittadini N, Alvaro R, Moretti A, Calafiore D, Toro G, Gimigliano F, Resmini G, Brandi ML. Clinical guidelines for the prevention and treatment of osteoporosis: summary statements and recommendations from the Italian Society for Orthopaedics and Traumatology. *J Orthop Traumatol Off J Ital Soc Orthop Traumatol*. 2017;18(Suppl 1):3–36.
4. Mallon PWG. Aging with HIV: osteoporosis and fractures. *Curr Opin HIV AIDS*. 2014;9(4):428–435.
5. McComsey GA, Tebas P, Shane E, Yin MT, Overton ET, Huang JS, Aldrovandi GM, Cardoso SW, Santana JL, Brown TT. Bone Disease in HIV Infection: A Practical Review and Recommendations for HIV Care Providers. *Clin Infect Dis Off Publ Infect Dis Soc Am*. 2010;51(8):937–946.
6. Dong HV, Cortés YI, Shiau S, Yin MT. Osteoporosis and fractures in HIV/hepatitis C virus coinfection: a systematic review and meta-analysis. *AIDS Lond Engl*. 2014;28(14):2119–2131.
7. Prieto-Alhambra D, Güerri-Fernández R, De Vries F, Lalmohamed A, Bazelier M, Starup-Linde J, Diez-Perez A, Cooper C, Vestergaard P. HIV infection and its association with an excess risk of clinical fractures: a nationwide case-control study. *J Acquir Immune Defic Syndr* 1999. 2014;66(1):90–95.
8. Rodger AJ, Lodwick R, Schechter M, Deeks S, Amin J, Gilson R, Paredes R, Bakowska E, Engsig FN, Phillips A, INSIGHT SMART, ESPRIT Study Groups. Mortality in well controlled HIV in the continuous antiretroviral therapy arms of the SMART and ESPRIT trials compared with the general population. *AIDS Lond Engl*. 2013;27(6):973–979.
9. Hileman CO, Labbato DE, Storer NJ, Tangpricha V, McComsey GA. Is bone loss linked to chronic inflammation in antiretroviral-naïve HIV-infected adults? A 48-week matched cohort study. *AIDS Lond Engl*. 2014;28(12):1759–1767.
10. Collin F, Duval X, Le Moing V, Piroth L, Al Kaied F, Massip P, Villes V, Chene G, Raffi F, ANRS CO8 APROCO-COPILOTE study group. Ten-year incidence and risk factors of bone fractures in a cohort of treated HIV1-infected adults. *AIDS Lond Engl*. 2009;23(8):1021–1024.
11. Comisia Nationala de Lupta Anti-SIDA - Date statistice [Internet]. [cited 2018 Dec 25]. Available from: http://www.cnlas.ro/com_jce/date-statistice.html
12. Grigorie D, Coles D, Socaliuc A. Trabecular bone score (TBS) has a poor discriminative power for vertebral fractures in 153 Romanian patients with primary hyperparathyroidism. *Acta Endocrinol Buchar*. 2018;14(2):208–212.
13. Yilmaz O, Mevsim V, Kuruoglu E. Development of clinical risk assessment tool for osteoporosis (osteoriskapp) using a syndromic approach. *Acta Endocrinol (Buc)*. 2018;14(1):133–141.
14. Chițu-Tișu C-E, Barbu E-C, Lazăr M, Ion DA, Bădăraș IA. Low bone mineral density and associated risk factors in HIV-infected patients. *Germes*. 2016;6(2):50–59.
15. De Laet C, Kanis JA, Odén A, Johanson H, Johnell O, Delmas P, Eisman JA, Kroger H, Fujiwara S, Garnero P, Mc Closkey EV, Mellstrom D, Melton LJ 3rd, Meunier PJ, Pols HA, Reeve J, Silman A, Tenenhouse A. Body mass index as a predictor of fracture risk: a meta-analysis. *Osteoporos Int J Establ Result Coop Eur Found Osteoporos Natl Osteoporos Found USA*. 2005;16(11):1330–1338.
16. Oldroyd A, Dubey S. The association between bone mineral density and higher body mass index in men. *Int J Clin Pract*. 2015;69(1):145–147.
17. Joakimsen RM, Fønnebo V, Magnus JH, Tollan A, Sjøgaard AJ. The Tromsø Study: body height, body mass index and fractures. *Osteoporos Int J Establ Result Coop Eur Found Osteoporos Natl Osteoporos Found USA*. 1998;8(5):436–442.
18. Pinnetti C, Federico L, Lorenzini P, Domenico C, Rita B, Laura L, Zaccarelli M, Cicalini S, Libertone R, Giannetti A, Mosti S, Rizzi EB, Antinori A, Ammassari A. Relationship between body mass index and bone mineral density in HIV-infected patients referred for DXA. *J Int AIDS Soc*. 2014;17(4 Suppl 3):19569.
19. Munteanu D, Negru A, Mihăilescu R, Tilișcan C, Tudor AM, Lazăr M, Aramă ȘS, Ion D, Popescu C, Aramă V. Evaluation of Bone Mineral Density and Correlations with Inflammation Markers in Romanian HIV-Positive Patients Undergoing Combined Antiretroviral Therapy. *Farmacia*. 2017;65:114–119.
20. Reid IR, Bolland MJ, Grey A. Effects of vitamin D supplements on bone mineral density: a systematic review and meta-analysis. *Lancet Lond Engl*. 2014;383(9912):146–155.
21. Poiana C, Capatina C, Cercel AS, Sandulescu O, Streinu Cercel A. Hypovitaminosis D in HIV-infected patients. *Acta Endocrinol (Buc)*. 2019;15(1):102–106.
22. Hileman CO, Overton ET, McComsey GA. Vitamin D and Bone Loss in HIV. *Curr Opin HIV AIDS*. 2016;11(3):277–284.
23. Cervero M, Agud JL, García-Lacalle C, Alcázar V, Torres R, Jusdado JJ, Moreno Guillen S. Prevalence of vitamin D deficiency and its related risk factor in a Spanish cohort of adult HIV-infected patients: effects of antiretroviral therapy. *AIDS Res Hum Retroviruses*. 2012;28(9):963–971.
24. Lerma E, Molas ME, Montero MM, Guellar A, González A, Villar J, Diez A, Knobel H. Prevalence and Factors Associated with Vitamin D Deficiency and Hyperparathyroidism in HIV-Infected Patients Treated in Barcelona. *Isrn Aids*. 2012;2012:485307.
25. Theodorou M, Sersté T, Van Gossom M, Dewit S. Factors associated with vitamin D deficiency in a population of 2044 HIV-infected patients. *Clin Nutr Edinb Scotl*. 2014;33(2):274–279.
26. Erlandson KM, Lake JE, Sim M, Falutz J, Prado CM, da Silva ARD, Brown TT, Guaraldi G. Bone Mineral Density Declines Twice as Quickly Among HIV-Infected Women Compared to Men. *J Acquir Immune Defic Syndr* 1999. 2018;77(3):288–294.
27. Atteritano M, Mirarchi L, Venanzi-Rullo E, Santoro D, Iaria C, Catalano A, Lasco A, Arcoraci V, Lo Gullo A, Bitto A, Squadrito F, Cascio A. Vitamin D Status and the Relationship with Bone Fragility Fractures in HIV-Infected Patients: A Case Control Study. *Int J Mol Sci [Internet]*. 2018 [cited 2019];19(1).
28. Kruger MJ, Nell TA. Bone mineral density in people living with HIV: a narrative review of the literature. *AIDS Res Ther [Internet]*. 2017 [cited 2019];14.
29. Gohda J, Ma Y, Huang Y, Zhang Y, Gu L, Han Y, Li T, Gao B, Gao GF, Inoue J, Iwamoto A, Ishida T. HIV-1 replicates in human osteoclasts and enhances their differentiation *in vitro*. *Retrovirology [Internet]*. 2015 [cited 2019];12.
30. Brown TT, Qaqish RB. Antiretroviral therapy and the prevalence of osteopenia and osteoporosis: a meta-analytic review. *AIDS Lond Engl*. 2006;20(17):2165–2174.
31. Brown TT, McComsey GA, King MS, Qaqish RB, Bernstein BM, da Silva BA. Loss of bone mineral density after antiretroviral therapy initiation, independent of antiretroviral regimen. *J Acquir Immune Defic Syndr* 1999. 2009;51(5):554–561.
32. Moyle GJ, Stellbrink H-J, Compston J, Orkin C, Arribas JR, Domingo P, Granier C, Pearce H, Sedani S, Gartland M, ASSERT Team. 96-Week results of abacavir/lamivudine *versus* tenofovir/emtricitabine, plus efavirenz, in antiretroviral-naïve, HIV-1-

infected adults: ASSERT study. *Antivir Ther.* 2013;18(7):905–913.

33. Carr A, Miller J, Eisman JA, Cooper DA. Osteopenia in HIV-infected men: association with asymptomatic lactic acidemia and lower weight pre-antiretroviral therapy. *AIDS Lond Engl.* 2001;15(6):703–709.

34. Duvivier C, Kolta S, Assoumou L, Ghosn J, Rozenberg S, Murphy RL, Katlama C, Costagliola D, ANRS 121 Hippocampe study group. Greater decrease in bone mineral density with protease inhibitor regimens compared with nonnucleoside reverse transcriptase inhibitor regimens in HIV-1 infected naive patients. *AIDS Lond Engl.* 2009;23(7):817–824.

35. Yin MT, Modarresi R, Shane E, Santiago F, Ferris DC, McMahon DJ, Zhang CA, Cremers S, Laurance J. Effects of HIV infection and antiretroviral therapy with ritonavir on induction of osteoclast-like cells in postmenopausal women. *Osteoporos Int J Establ Result Coop Eur Found Osteoporos Natl Osteoporos Found USA.* 2011;22(5):1459–1468.

36. Nylén H, Habtewold A, Makonnen E, Yimer G, Bertilsson L, Burhenne J, Diczfalusy U, Aklillu E. Prevalence and risk factors for efavirenz-based antiretroviral treatment-associated severe vitamin D deficiency. *Medicine (Baltimore) [Internet].* 2016 [cited 2019];95(34).