Research article

Bone mineral density abnormalities in HIV infected patients and HIV negative respondents at Mbagathi Hospital using calcaneal quantitative ultrasound

Abdullahi AM, Oyoo GO, Sheikh AM, Maritim MC, Kwasa JK

Department of Clinical Medicine and Therapeutics, School of Medicine, College of Health Sciences, University of Nairobi, P O Box 19676-00202, Nairobi, Kenya

Corresponding author:
Dr AM Abdullahi.
Email: Adrabdilahi@gmail.com

Background: Osteoporosis is a systemic skeletal disorder characterized by low bone mass and micro-architectural deterioration of bone tissue, with a consequent increase in bone fragility and fracture. Use of Highly Active Anti-Retroviral Therapy (HAART) has been associated with prolonged survival and consequently with an increase in the prevalence of decreased bone mineral density. Quantitative Ultrasound (QUS) is gaining popularity as an appropriate tool for determination of bone mineral density profiles in resource- poor settings.

Objectives: To determine and compare the difference in the prevalence of Bone Mineral Density (BMD) abnormalities using quantitative calcaneal ultrasound between HIV infected patients on a TDF based first-line regime for at least one year, HAART-naive HIV positive patients in Mbagathi Comprehensive Care Clinic (CCC) and a HIV negative control group seen at the Mbagathi Voluntary Counselling and Testing Centre (VCT). To describe the occurrence of traditional risk factors associated with decreased BMD in the above populations (oral corticosteroid use, smoking, alcohol, previous bone fracture, body mass index and physical inactivity).

Methods: This was a cross-sectional comparative group descriptive study of HIV positive adult patients on TDF based first-line regime (exposed), HIV positive HAART- naive adult patients (unexposed) and HIV negative adult group (control) at Mbagathi Hospital. Random sampling was used to recruit 315 participants (105 in each arm). An interviewer administered questionnaire was used to document risk factors for low BMD. Quantitative ultrasound bone mineral density was done using a heel ultrasonic gel- coupled QUS system, the Sunlight Mini Omni (Beam Med Ltd, Israel).

Results: The prevalence of osteoporosis among HIV positive respondents on HAART was significantly higher (58.1%) compared to HIV positive respondents not on HAART (32.6%) (Z-test p-value = 0.001) and HIV negative respondents (9.3%) (Z-test p-value = 0.001). Older patients had lower levels of BMD (i.e. more negative BMD. p-value = 0.032). HIV positive respondents on HAART had lower BMI than HAART naïve and HIV negative individuals (23.6%, 24.8% and 26.1% respectively). There was a significant positive correlation between T-score and BMI (p-value 0.043). There was no significant correlation between T-score and the other traditional risk factors (oral corticosteroid use, smoking, alcohol use, history of bone fractures and physical activity).

Conclusions: Use of TDF based HAART regimes is associated with higher rates of osteoporosis compared to HAART naïve and HIV negative populations which may be partly mediated by lower Body Mass Index (BMI).

Introduction

Human Immune-Deficiency Virus (HIV) infection is one of the heaviest infectious disease burdens afflicting sub-Saharan countries. Kenya has the fourth-largest HIV epidemic in the world and in 2012, an estimated 1.6 million people were living with HIV, and roughly 57,000 people died from AIDS-related illnesses[1,2]. Since 2008, the expansion of ART services throughout the national healthcare system had increased the number of adults on treatment from 64% to 80% in 2013[3].

Use of Highly Active Anti-retroviral Therapy (HAART) has been associated with viral suppression and improved patient survival. With prolonged life, the prevalence of osteoporosis and osteopenia increases due to differential bone remodeling associated with aging[4,5]. HIV causes osteopenia through cytokine and inflammatory- mediated pathways[6,7]. Highly Active Anti-Retroviral Therapy (HAART) drugs have been associated with decreased Bone Mineral Density

(BMD) especially Tenofovir Disoproxil Fumarate (TDF) and Protease Inhibitor (PI) based regimens. This is probably through the effect of these medications on cellular DNA synthesis and gene expression involved in bone re-modelling[8].

The WHO recommends the use of Dual Energy X-ray Absorptiometry (DXA, previously DEXA) method to determine BMD levels, and has provided guidance on classifying the levels into clinically relevant outcomes depending on the number of Standard Deviations (SDs) below the mean BMD for a healthy, young (25–35 years of age), sex- and ethnicity-matched reference population (T-score).

Other methods used to determine bone mineral density include Quantitative Computer Tomography (QCT) and Quantitative Ultrasound (QUS). Both DXA and QCT involves utilization of specialized equipment, generate ionizing radiation, are expensive and require relative expertise.

Quantitative calcaneal ultrasonography offers several benefits. It is cheaper and more portable than DEXA, there is no exposure to ionizing radiation[10] and is as effective as DEXA at predicting femoral neck, hip, and spine osteoporotic fractures [4,11].

Materials and Methods

This was a hospital based study carried out over a fifteen week period between 4th May and 14th August 2015 at Mbagathi Hospital CCC and VCT Centre. A random generation table was used to select participants from the clinic and VCT Centre of which 105 participants were selected in each arm (total of 315) after satisfying the inclusion criteria for each arm. A questionnaire was administered which captured demographic data, duration of HIV/HAART use and occurrence of traditional risk factors among the respondents. QUS bone mineral density was assessed using a heel ultrasonic gel-coupled QUS system, the Sunlight Mini Omni (Beam Med Ltd, Israel). The participants were asked to remove their shoes and stand with one foot on the ultrasound machine. Three repeated measurements with repositioning was performed on the same foot for all participants. BUA was expressed as a T-score (standard deviations from the mean value in normal young individuals of the same sex) using the manufacturer's age- and sex-specific reference data. A bone densitometry form was filled for each participant showing their bone mineral density.

Results

In this fifteen week study, 105 patients were recruited in the HAART naïve and TDF based HAART regime arms (from Mbagathi CCC) and105 individuals who were HIV negative were recruited from the Mbagathi VCT Centre (Figure 1).

Figure 1: Recruitment process

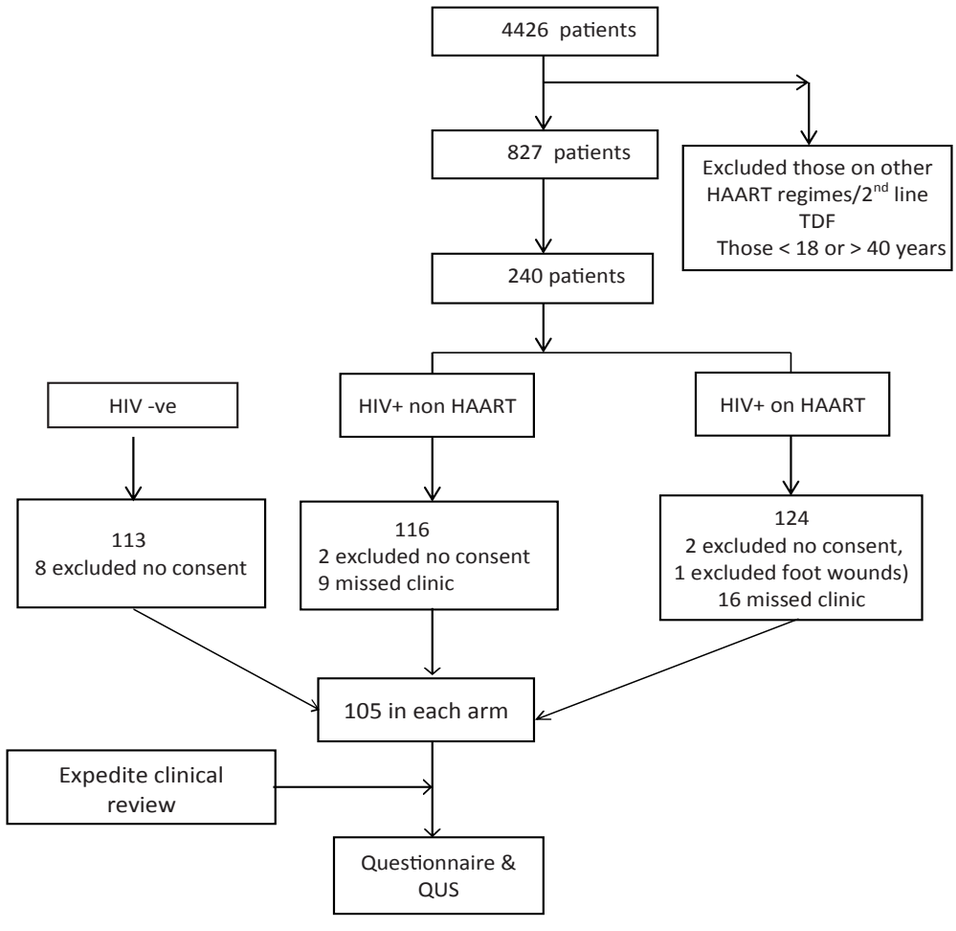
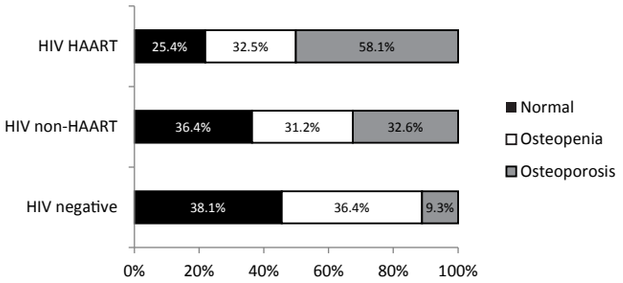


Table 1: Socio-demographic characteristics among the comparative arms

Characteristic	Categories	HIV negative No. (%)	HIV non-HAART No. (%)	HIV on HAART No. (%)	Total (%)
Age (years)	18-22	15(14.1)	13(12.5)	7(6.8)	43.5% (29-28 years)
	23-28	46(44.7)	22(21.1)	15(14.2)	
	29-34	31(29.5)	43(40.6)	31(29.5)	
	35-40	12(11.6)	27(26.8)	52(49.5)	
Gender	Male	48(45.7)	46(43.3)	44(41.4)	137(43.5)
	Female	57(54.3)	59(56.7)	61(58.6)	178(56.5)
Marital status	Married	14(13.2)	32(30.2)	60(56.6)	106(33.7)
	Single	83(52.2)	53(33.3)	23(14.5)	159(50.5)
	Divorced	8(19.5)	18(43.9)	15(36.6)	41(13.0)
	Widowed	0(.0)	2(22.2)	7(77.8)	9(2.9)
Residence	Rural	8(44.4)	1(5.6)	9(50.0)	18(5.7)
	Urban	97(32.7)	104(35.0)	96(32.3)	297(94.3)
Highest education level	None	0(.0)	1(100.0)	0 (.0)	1(0.3)
	Primary	21(20.6)	46(45.1)	35(34.3)	102(32.4)
	Secondary	21(17.1)	50(40.7)	52(42.3)	123(39.0)
	Tertiary	63(60.8)	8(9.0)	18(20.2)	89(28.3)
Occupation	Unemployed	12(25.0)	23(47.9)	13(27.1)	48(15.2)
	Student	60(85.7)	8(11.4)	2(2.9)	70(22.2)
	Self employed	21(17.6)	45(37.8)	53(44.5)	119(37.8)
	Civil servant	3(25.0)	4(33.3)	5(41.7)	12(3.8)
	Other	9(13.6)	25(37.9)	32(48.5)	66(21.0)
Income level per month (Kshs)*	Below 2500	55(51.4)	39(36.4)	13(12.1)	107(34.0)
	2500 - 5000	22(36.1)	25(41.0)	14(23.0)	61(19.4)
	5000 - 10000	16(23.2)	22(31.9)	31(44.9)	69(21.9)
	10000 - 30000	10(14.9)	18(26.9)	39(58.2)	67(21.3)
	>30000	2(18.2)	1(9.1)	8(72.7)	11(3.5)

*1US $ = Kshs 100

Figure 2: Prevalence of BMD abnormalities among comparative arms



The mean T-score of HIV negative respondents was -1.197(±0.168) compared to mean T-scores of -1.311(±0.184) and -1.740(±0.231) in the HIV positive HAART naïve and HIV positive on HAART respondents respectively. There was a significant difference in mean T-score between the comparative arms (ANOVA p-value < 0.001).

Table 2: Socio-demographic characteristics and BMD

Characteristics	Categories	Normal BMD	Ostopenia	Osteoporosis	P- value
Age (years)	18-22	48.1	30.2	21.7	0.032
	23-28	44.5	29.3	26.2	
	29-34	38.4	33.0	28.6	
	35-40	22.0	31.9	46.1	
Gender	Male	39.7%	50.9%	9.5%	0.257
	Female	36.2%	47.7%	16.1%	
Marital status	Married	32.1%	46.2%	21.7%	0.086
	Single	39.6%	52.8%	7.5%	
	Divorced	36.6%	46.3%	17.1%	
	Widowed	66.7%	22.2%	11.1%	
Residence	Rural	33.3%	38.9%	27.8%	0.195
	Urban	37.7%	49.5%	12.8%	
Highest education level	None	100.0%	0.0%	0.0%	0.756
	Primary	36.3%	47.1%	16.7%	
	Secondary	39.8%	48.0%	12.2%	
	Tertiary	34.8%	52.8%	12.4%	
Occupation	Unemployed	41.7%	45.8%	12.5%	0.280
	Student	38.6%	55.7%	5.7%	
	Self employed	37.0%	46.2%	16.8%	
	Civil servant	58.3%	25.0%	16.7%	
	Other	30.3%	53.0%	16.7%	
Income level per month (Kshs)*	Below 2500	40.2%	49.5%	10.3%	0.798
	2500 – 5000	34.4%	47.5%	18.0%	
	5000 – 10000	39.1%	44.9%	15.9%	
	10000 – 30000	37.3%	50.7%	11.9%	
	>30000	18.2%	63.6%	18.2%	

* 1 US $ = Kshs 100

There was significant negative correlation between T-score and age of the respondents (Spearman r = -.121, p-value = 0.032). This implied that older patients were associated with lower levels of BMD (i.e. more negative BMD). There was no significant association between low BMD and marital status (p-value = 0.086), gender (p-value = 0.257), residence (p-value = 0.195), highest education level (p-value = 0.756), occupation (p-value = 0.280) and income level per month (p-value = 0.798).

Table 3: Traditional risk factors distribution among the comparative arms

Risk factor	HIV Negative	HIV Non HAART	HIV on HAART
Oral corticosteroid use	3.8%	1.9%	0%
Current smokers (n=11)	6.7%	1.9%	1.9%
Used to smoke (n=33)	8.6%	7.6%	15.2%
Alcohol intake (n=160)			
Once monthly or less (n=122)	50.4%	45.7%	20.0%
Weekly(n=35)	19.0%	8.6%	5.7%
Daily (n=3)	1.9%	0.9%	0.0%
Sustained bone fracture	16.1%	20.0%	13.3%
Physical activity levels			
Vigorous	22.8%	13.3%	14.2%
Moderate	43.9%	35.3%	45.7%
minimal	33.3%	51.4%	40.0%
BMI(Kg/m²)	26.1	24.8	23.6

Body Mass Index (BMI): There was a significant difference in BMI among the comparative arms (ANOVA p-value < 0.001) with HIV negative patients having significantly the highest BMI on average followed by HIV non-HAART then HIV HAART patients. There was significant positive correlation between T-score and BMI (Pearson R = 0.085, p-value = 0.043).

Table 4: Multivariate analysis

Variable	Significance	Odds Ratio	95% C.I. for Odds Ratio	
			Lower	Upper
Age in years	0.364	1.017	.981	1.053
BMI (Ref≥25)	0.046	1.962	1.607	2.225

Multivariate analysis: Factors that were significant at the bivariate stage (age and BMI) underwent multivariate analysis to identify the predictors of decreased bone mineral density. BMI was the only risk factor identified as significant to predict occurrence of decreased bone mineral density at (p-value of 0.046) (Table 4).

Discussion

Our study populations were adults between 18-40 years old with a female preponderance of 56.5%. This was in keeping with our national HIV demographics[1,3]. Though we did not match age and gender among the comparative arms (resource constraints), our study showed a significant negative correlation between T-score and age of the respondents. This implied that older respondents were associated with lower Bone Mineral Density (BMD) levels. John *et al*[13] attributed this to age related changes in bone homeostasis and increased bone fragility.

Our study showed that the prevalence of osteoporosis among HIV positive respondents on HAART was significantly higher as compared to HIV positive respondents not on HAART and HIV negative respondents. Our study showed 58.1%, 32.6% and 9.3% of those on HAART, HIV positive HAART naïve and HIV negative respondents respectively were osteoporotic. This reflects a six-fold higher prevalence of osteoporosis between HIV infected individuals and the HIV negative controls. In other studies comparing HIV infected to uninfected populations the T-score difference between the two groups (HIV infected and uninfected) varied from 2.5-fold to 10-fold[14-17].

Flöter *et al*[18] in a review of six comparative studies between DEXA and QUS of the calcaneus concluded that the QUS sensitivity (79% to 93%) and specificity (28% to 90%) had wide variations which may lead to over or under diagnosis of bone density abnormalities at the WHO recommended T-score cut off of ≥ 2.5. He also noted that the diagnostic accuracy may be improved by varying the cut off T-score. This could partly explain the high rates of bone mineral density abnormalities in our study.

QUS and DEXA simply measures different bone characteristics (bone quality and bone quantity respectively). QUS can thus determine the strength of bone micro-architecture which may be associated with impaired bone structure with a higher risk of fractures and lower BMD[19]. QUS parameters including Speed of Sound (SOS), Bone Ultrasound Attenuation (BUA) and bone stiffness provide additional, specific and different information which may be useful in the integrative assessment of bone health[20].

It is also important to note that QUS has been extensively researched in large prospective studies and meta-analyses and has demonstrated comparable utility and diagnostic accuracy to DEXA at hip and non-spinal bone sites [21-27].

In our study, the prevalence of osteopenia was 32.5%, 31.2% and 36.4% in those HAART, HIV positive HAART naïve and HIV negative respondents respectively. This was in keeping with a meta-analysis of 37 studies by Brown *et al*[4] which showed significant heterogeneity between the studies for reduced BMD with osteopenia of between 4% - 56% in the HIV negative respondents and 13% - 62% in the HIV positive respondents on HAART. Poor dietary intake of calcium rich foods especially in childhood and adolescence could explain the similar rates of osteopenia across the comparative arms[12].

Several studies[4,28] have shown the association of Tenofovir Disoproxil Fumarate (TDF) with nephrotoxicity and hypophosphatemia due to renal tubular dysfunction leading to impaired Vitamin D metabolism which may determine low BMD in HIV patients.

HIV infection has been associated with decreased BMD mainly through cytokine dysregulation and impaired Vitamin D metabolism[29-32]. Thus the longer duration of living with HIV may be associated with low BMD[33]. We did not find significant association between T-score values and length of living with HIV which could be attributed to the relatively short duration of living with HIV among the respondents, with a mean duration of 4.8 years. Body Mass Index (BMI) was 23.6%, 24.8% and 26.1% among HIV positive on HAART, HIV positive HAART naïve and HIV negative respondents respectively. We did find a significant negative correlation between T-score and BMI. Respondents with low BMI were likely to have lower BMD values.

The difference in Bone Mineral Density (BMD) abnormalities was in part, related to the difference in Body Mass Index (BMI) between those on HAART and HIV negative respondents. Bone mass is known to be positively correlated with BMI, as an indicator of muscular mass, and HIV infected individuals usually have lower body weight compared with uninfected persons[34,35]. A meta-analysis by Bolland *et al*[36] showed that, after adjustment for weight, residual between-group differences in bone mineral density were small (2.2-4.7%) and unlikely to be clinically significant.

Poor dietary intake of milk especially in childhood and adolescence has been associated with low bone mineral density [12,37]. This could partly explain low BMD in our study participants who are from a low socioeconomic catchment area.

Most longitudinal studies involving HAART-naïve individuals showed that bone mineral density declined by 2-6% within 24-48 weeks after initiation of HAART[38-41]. Thereafter, bone mineral density values remained stable or

even increased slightly[42]. We did not find any association between QUS bone mineral density and duration of treatment with HAART. This could be attributed to the fact that majority of the respondents (68%) had received HAART for at least 45 months.

Persons who consume moderate amounts of alcohol have a lower risk of hip fractures compared to heavy drinkers[43]. We did not find significant difference in bone mineral density in the respondents who consumed alcohol. This could be due to the fact that 50.8% of the total respondents in our study took alcohol of whom76.3% consumed alcohol once a month or less and only 0.9% of the study participants who consumed alcohol daily.

Karnis *et al*[44] in a multi-center prospective study concluded that the risk of fractures is greater for smokers and those with a history of smoking compared to non-smokers. We did not find significant difference in BMD between those who smoked, had prior history of smoking and non-smokers. There was also no significant correlation between pack years smoked and BMD. This could be attributed to the fact that only 3.5% of the total respondents smoked, with 2.7 average pack years and a study population of young adults.

We did not find any association between oral corticosteroid use with decreased BMD. This could be attributed to the low number of respondents on oral corticosteroids (5.7%), though duration of steroid use and preventive measures against steroid induced osteoporosis (vitamin D and calcium supplementation use) was not assessed. Further studies are required to determine the relationship of duration of corticosteroid use with BMD in the HIV population. 43.9%, 45.3% and 45.7% of HIV negative, HAART naïve and those on TDF based regime respectively were involved in moderate physical activities. We did not find any difference in BMD values in the intensity levels of physical activity among the comparative arms. This could be attributed to the fact that the respondents were from a low socio-economic background (53.4% earned <kshs 5000 or US$50/month) and could not afford public transport and would therefore walk to work.

We have shown, in an African setting, that HIV infected patients on a TDF based regime have reduced Quantitative Ultrasound bone mineral density in comparison to HAART naïve and HIV negative populations. However, the clinical significance of this result in terms of osteoporosis remains unknown, since we could not use the validated reference method by WHO for bone mineral density assessment.

References

1. United Nations programme on HIV/AIDS (UNAIDS). Report on the global AIDS epidemic. 2013.
2. UNGASS, *Kenya Aids Progress Report 2014. Progress towards zero*. 2014.
3. NASCOP, N.a., *Kenya AIDS Epidemic update 2011* 2012: Nairobi, Kenya.
4. Brown TT, Qaqish RB. Antiretroviral therapy and the prevalence of osteopenia and osteoporosis: a meta-analytic review. *AIDS,* 2006; **20**(17): 2165-2174.
5. McComsey GA, Tebas P, Shane E, Yin MT. Bone disease in HIV infection: a practical review and recommendations for HIV care providers. *Clin Infect Dis*. 2010; **51**(8): 937-946.
6. Triant VA, Brown TT, Lee H, Grinspoon SK. Fracture prevalence among human immunodeficiency virus (HIV)-infected versus non-HIV-infected patients in a large U.S. healthcare system. *J Clin Endocrinol Metab*. 2008; **93**(9): 3499-504.
7. Womack JA, Goulet JL, Gibert C, Brandt C, Chang CC, Gulanski B, *et al*. *PLoS One*. 2011; **6**(2): e17217.
8. Fux CA, Rauch A, Simcock M. Tenofovir use is associated with an increase in serum alkaline phosphatase in the Swiss HIV Cohort Study. *Antivir Ther*. 2008; **13**(8): 1077-1082.
9. Consensus development conference: diagnosis, prophylaxis, and treatment of osteoporosis. *Am J Med*. 1993; **94**(6): 646-650.
10. Prins SH, Jorgensen HL, Jorgensen LV, Hassager C. The role of quantitative ultrasound in the assessment of bone: a review. *Clin Physiol*. 1998; **18**(1): 3-17.
11. El-Desouki, MI, Sherafzal MS, Othman SA. Comparison of bone mineral density with dual energy x-ray absorptiometry, quantitative ultrasound and single energy x-ray absorptiometry. *Saudi Med J*. 2005; **26**(9): 1346-50.
12. Constant D, Rosenberg L, Zhang Y, Cooper D, Kalla A, Micklesfield L, *et al*. Quantitative ultrasound in relation to risk factors for low bone mineral density in South African pre-menopausal women. *Arch Osteoporos*. 2009; **4**(1-2): 55-65.
13. John A, Kanis EVMC. Risk factors in osteoporosis. *Maturitas J Europ Menopause and Andropause Society.* 1998; **30**(3): 229 - 233.
14. Bruera D, *et al*. Decreased bone mineral density in HIV-infected patients is independent of antiretroviral therapy. *Aids*. 2003; **17**(13): 1917-1923.
15. Tebas P, Powderly W, Yarasheski K. Response to 'Accelerated bone mineral loss in HIV-infected patients receiving potent antiretroviral therapy' by Drs Weil and Lenhard. *Aids*. 2000; **14**(15): 2417.
16. Amiel C, Ostertag A, Slama L, Baudoin C, N'Guyen T, Lajeunie E, *et al*. BMD is reduced in HIV-infected men irrespective of treatment. *J Bone Miner Res.* 2004; **19**(3): 402-409.
17. Arnsten JH, Freeman R, Howard AA, Floris-Moore M, Santoro N, Schoenbaum EE. HIV infection and bone mineral density in middle-aged women. *Clin Infect Dis*. 2006; **42**(7): 1014-1020.
18. Flöter M, Bittar CK, Zabeu JL, *et al*. Review of comparative studies between bone densitometry and quantitative ultrasound of the calcaneus in osteoporosis. *Acta Rheumatol Port.* 2011; **36**(4):327-335.
19. Stagi S, Cavalli L, Bertini F, *et al*. Cross-sectional and longitudinal evaluation of bone mass and quality in children and young adults with Juvenile Onset Systemic Lupus Erythematosus: role of bone mass determinants analysed by DXA, PQCT and QUS. *Lupus*. 2014; **23**: 57-68.

20. Alberto C, Davide G, Davide D, *et al*. Calcaneal quantitative ultrasound (QUS) and dual X - ray absorptiometry (DXA) bone analysis in adult HIV-positive patients. *New Microbiologica*. 2015; **38**: 345-356.
21. Bauer DC, Gluer CC, Cauley JA, *et al*. Broadband ultrasound attenuation predicts fractures strongly and independently of densitometry in older women. A prospective study. Study of Osteoporotic Fractures Research Group. *Arch Intern Med*. 1997; **157**(6): 629-634.
22. Hans D, Dargent-Molina P, Schott AM, Sebert JL, Cormier C, Kotzki PO, *et al*. Ultrasonographic heel measurements to predict hip fracture in elderly women: the EPIDOS prospective study. *Lancet*. 1996; **348**(9026): 511-514.
23. Khaw KT, Reeve J, Luben R. Prediction of total and hip fracture risk in men and women by quantitative ultrasound of the calcaneus: EPIC-Norfolk prospective population study. *Lancet*. 2004; **363**(9404): 197-202.
24. Pluijm SM, Graafmans WC, Bouter LM, Lips P. Ultrasound measurements for the prediction of osteoporotic fractures in elderly people. *Osteoporos Int*. 1999; **9**(6): 550-556.
25. Nayak S, Olkin I, Liu H, Grabe M, Gould MK, Allen IE, *et al*. Meta-analysis: accuracy of quantitative ultrasound for identifying patients with osteoporosis. *Ann Intern Med*. 2006; **144**(11): 832-841.
26. Blanckaert F, Cortet B, Coquerelle P, Flipo RM, Duquesnoy B, Marchandise X, *et al*. Contribution of calcaneal ultrasonic assessment to the evaluation of postmenopausal and glucocorticoid-induced osteoporosis. *Rev Rhum Engl Ed*. 1997; **64**(5): 305-313.
27. Daens S, Peretz A, de MV, Moris M, Bergmann P. Efficiency of quantitative ultrasound measurements as compared with dual-energy X-ray absorptiometry in the assessment of corticosteroid-induced bone impairment. *Osteoporos Int*. 1999; **10**(4): 278-283.
28. Duvivier C, Kolta S, Assoumou L, Ghosn J, Rozenberg S, Murphy RL, *et al*. Greater decrease in bone mineral density with protease inhibitor regimens compared with nonnucleoside reverse transcriptase inhibitor regimens in HIV-1 infected naive patients. *Aids*. 2009; **23**(7): 817-824.
29. Haug C, Muller F, Aukrust P, Froland SS. Subnormal serum concentration of 1,25-vitamin D in human immunodeficiency virus infection: Correlation with degree of immune deficiency and survival. *J Inf Dis*. 1994; **169**(4): 889-893.
30. Haug C, Aukrust P, Haug E, Morkrid L, Muller F, Froland S. Severe deficiency of 1,25-dihydroxyvitamin D3 in human immunodeficiency virus infection: association with immunological hyperactivity and only minor changes in calcium homeostasis. *J Clin Endocrinol Metab*. 1998; 83(11): 3832-3838.
31. Haug CJ, Aukrust P, Lien E, Muller F, Espevik T, Froland S. Disseminated mycobacterium avium complex infection in AIDS: Immunopathogenic significance of an activated tumor necrosis factor system and depressed serum levels of 1,25 dihydroxyvitamin D. *J Inf Dis*. 1996; **173**(1): 259-262.
32. Dusso AVM, Powderly WG, Yarashesky KE, Tebas P. Protease inhibitors inhibit in vitro conversion of 25[OH]-viamin D to 1, 25[OH]2-vitamin D. *Antivir Ther*. 2000; **5**(Suppl 5): 19.
33. Knobel H, Guelar A, Vallecillo G, *et al*. Osteopenia in HIV-infected patients: is it the disease or is it the treatment? *Aids*. 2001; 15(6): 807-808.
34. Snelling ACC, Schaeffer M. Modifiable and non-modifiable factors associated with osteoporosis in postmenopausal women: results from the Third National Health and Nutritional Survey, 1988 - 94. *Women Health Gender Based Med*. 2001; **10**(1): 57-65.
35. Ravn P, Cizza G, Bjarson N, Thompson D, Daley M, Wasnich R, *et al*. Low body mass index is an important risk factor for low bone mass and increased bone loss in early postmenopausal women. Early Postmenopausal Intervention Cohort (EPIC) study group. *J Bone Miner Res*. 1999; **14**(9): 1622-1627.
36. Bolland MJ, Grey AB, Gamble GD, *et al*. Low body weight mediates the relationship between HIV infection and low bone mineral density: a meta-analysis. *J Clin Endocrinol Metab*. 2007; **92**: 4522-4528.
37. Karina MB, Hillary V.K, *et al*. Association between alcohol consumption and both osteoporotic fracture and bone density. *Am J Med*. 2008; **121**(5):406-418.
38. Briot K, Kolta S, Flandre P, Boue F, Ngo Van P, *et al*. Prospective one-year bone loss in treatment-naive HIV+ men and women on single or multiple drug HIV therapies. *Bone*. 2011; **48**(5): 1133-1139.
39. Brown TT, McComsey GA. Association between initiation of antiretroviral therapy with efavirenz and decreases in 25-hydroxyvitamin D. *Antivir Ther*. 2010; **15**(3): 425-429.
40. Duvivier C, Kolta S, Assoumou L, Ghosn J, Rozenberg S, *et al*. Greater decrease in bone mineral density with protease inhibitor regimens compared with nonnucleoside reverse transcriptase inhibitor regimens in HIV-1 infected naive patients. *Aids*. 2009; **23**(7): 817-824.
41. Gallant J, Staszewski S, Pozniak A, DeJesus E, Suleiman J, Miller M, *et al*. Efficacy and safety of tenofovir DF vs stavudine in combination therapy in antiretroviral-naive patients: a 3-year randomized trial. *JAMA*. 2004; **292**(2): 191-201.
42. Nolan D, Upton R, McKinnon E, *et al*. Stable or increasing bone mineral density in HIV-infected patients treated with nelfinavir or indinavir. *Aids*. 2001; **15**(10): 1275-1280.
43. Karina MB, Hillary V.K, *et al*. Association between alcohol consumption and both osteoporotic fracture and bone density. *Am J Med*. 2008; **121**(5):406-418.
44. Kanis JA, Johnell O, Oden A, Johansson H, De Laet C, Eisman JA, *et al*. Smoking and fracture risk: a meta-analysis. *Osteoporos Int*. 2005; **16**:155-162.