



BONE MINERAL DENSITY AND CARDIOVASCULAR RISK: ASSOCIATION IN INDIVIDUALS LIVING WITH HIV

ORIGINAL ARTICLE

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ABSTRACT

Introduction: Osteoporosis and cardiovascular diseases are frequent diseases, with high morbidity and mortality, and occur early in patients living with HIV. This study aimed to evaluate the association between reduced bone mineral density and increased cardiovascular risk in people living with HIV. **Methods:** This is an analytical cross-sectional study, with 82 individuals, who underwent bone densitometry by imaging of the lumbar spine and femoral neck, TCD4+ cell count and laboratory tests to assess the metabolic profile. The cardiovascular risk was estimated by specific algorithm for PLHIV (reduced D:A:D). The software Epi-Info (version 7.2.4), Bioestat 5.3 and JAMOV 1.6.23 were used for data analysis. **Results:** It was found a high prevalence of osteopenia and osteoporosis (62.2%), high cardiovascular risk (high: 26.83%; very high 42.68%) and a high prevalence of associated risk factors (age, lipodystrophy, dyslipidemia and diabetes). An association was found between reduced bone density and high cardiovascular risk in both sexes ($p=0.0120$; OR: 3.9091), indicating that individuals with reduced bone mass are 3.90 times more likely to develop cardiovascular events than those with normal bone density. In addition, it was found that the reduction in the T-score of the femoral neck ($p=0.0212$) and lumbar spine ($p=0.0159$) was related to an increase in cardiovascular risk. Univariate ordinal logistic regression showed an independent association between osteoporosis and high cardiovascular risk ($p=0.042$). **Conclusions:** The results suggest that the reduction in bone mineral density is associated with a higher



cardiovascular risk in PLHIV and may predict higher morbidity and mortality in this population.

Keywords: Osteoporosis, Osteopenia, Cardiovascular Risk, HIV.

1. INTRODUCTION

Osteoporosis and Cardiovascular Diseases (CVD) are frequent diseases in the population, their incidence increases with age and have impact on morbidity and mortality and public health expenditures^{1,2}.

The Association Between Reduced Bone Mineral Density (BMD) and CVD has been described regardless of the patients' age³, as well as there is genetic evidence that calcified plaques may have some bone matrix proteins in their composition, demonstrating that these diseases have pathophysiological similarities. In addition to pathophysiology and epidemiology, osteoporosis and CVD also share risk factors: early menopause, smoking, vitamin D deficiency, physical inactivity and genetic factors⁴.

In People Living With HIV (PLHIV), the estimated prevalence of osteopenia/osteoporosis and CVD is 2-3 times higher in PLHIV. Chronic use of Antiretroviral Therapy (HAART) is associated with a greater decline in BMD and an increased risk of fractures⁵.

The correlation between CVD and reduced BMD in the general population is already consolidated in the literature, however, the same cannot be said in relation to the population living with HIV. That said, this study aims to evaluate the association between bone mineral density and cardiovascular risk in PLHIV associated with the use of HAART, as well as to identify risk factors associated with reduced BMD and cardiovascular risk. Therefore, the results of this research aim to assist the medical community and health services in incorporating protocols and clinical practices that aim to identify, treat and delay the progression of these pathologies, with a view to reducing cardiovascular mortality and improving the quality of life of PLHIV.



2. METHODS

2. 1 STUDY DELINEATION

This is a cross-sectional study, with a quantitative approach, carried out at the outpatient clinic of Endocrinology in patients living with HIV conducted in a tertiary care hospital in Pará, Brazil.

The study sampling was carried out by convenience sample selection, obtaining a total of 82 individuals treated at the outpatient clinic from January to October 2022. The inclusion criteria were adult individuals (>18 years of age), of both sexes, with positive serology to HIV, using HAART and who had bone densitometry and CD4+ T lymphocyte count results, who agreed to participate in the study by signing the Free and Informed Consent Form. Patients with bone densitometry results older than 24 months elapsed in relation to the interview date were excluded.

2. 2 DATA COLLECTION

The data collection utilized a form containing socio-demographics and clinical-epidemiological variables (risk factors for developing cardiovascular diseases, associated previous comorbidities, history of cardiovascular diseases in the family and osteoporosis).

Furthermore, the anthropometric data collected was: weight in kilograms, height in meters, Body Mass Index in kg/m² (BMI)⁶ and Abdominal Circumference (AC)⁷. The patients were also categorized based on presence or absence of clinical forms of lipodystrophy syndrome caused by HIV, through physical examination: lipoatrophy, lipo-hypertrophy, “mixed” lipodystrophy or no lipodystrophy⁸.

All patients underwent blood collection through peripheral venipuncture after 12 hours fasting period to dose blood glucose (mg/dl), HDL-cholesterol (mg/dl),



LDL-cholesterol (mg/dl) and triglycerides (mg/dl)⁹. Blood collection was carried out by nursing technicians and the blood samples were processed by the clinical analysis laboratory linked to the hospital where the study was carried out.

To estimate the cardiovascular risk of the individuals in the next 10 years, the score for cardiovascular risk assessment utilized was the reduced data collection on adverse effects of anti-HIV drugs (D: A:D), that takes into account the age, sex, current or previous smoking, family history of cardiovascular disease, diabetes mellitus, cd4 lymphocyte count, blood pressure levels and serum dosages of total cholesterol and HDL cholesterol¹⁰.

The BMD was obtained by the image originates from the Dual Emission X-ray Absorptiometry (DXA) of the Lumbar Spine (LS) and Femoral Neck (FN), using the Hologic method¹¹.

2. 3 STATISTICAL ANALYSIS

The data were organized in the Microsoft Excel 2010 program. The graphics and spreadsheets were constructed with the tools in Microsoft Word, Excel, Bioestat 5.5 and Epi-info (7.2.4 version). The statistical tests were performed with the aid of Bioestat 5.3 and JAMOV 1.6.23 software.

Quantitative variables were described by minimum, maximum, mean, median and standard deviation and qualitative variables by frequency and percentage. The independence or association between two categorical variables was tested by the Chi-Square test or Fisher's exact or G test.

To compare a numerical variable between two groups, the student's t-test or the nonparametric equivalent Mann-Whitney test was used. To perform the analysis of variance of quantitative variables with normal distribution, the ANOVA statistical test was used, and for those that did not have normal distribution, the Kruskal-Wallis Analysis of Variance test was used.

To evaluate the correlation between two nonparametric quantitative variables, the Spearman correlation test was performed. To evaluate the probability of the



occurrence of events associated with a dependent variable, univariate and multivariate ordinal regression tests were performed.

The references used for the logistic regression tests were: BMD (normal), sex (male); diabetes (not being diabetic). Results with $p \leq 0.05$ (bilateral) were considered statistically significant.

2. 4 ETHICAL ASPECTS

This study was approved by Research Ethics Committee of the Health Sciences Institute of UFPA, decision number 5.502.345, CAAE: 57926522.7.0000.0018. Written informed consent was obtained for participation in the study and use of the patient data for research and educational purposes. The procedures in the study follow the guidelines laid down in Declaration of Helsinki (1964).

3. RESULTS

The 82 individuals were included in the study. From the sociodemographic data, it was verified that there was a predominance of males (55.1%), with the majority age group being 50 to 69 years (58.55%). The mean age of the participants was 54.26 years (Table 3).

In this sample, the mean time of HIV infection and HAART use was 15.31 and 14.85 years, respectively. The highest frequency was in those who had more than 20 years of infection and use of ART (Table 1).

Table 1 - Sociodemographic and clinical characteristics of PLHIV under study, (Belém – PA, 2022)

Variables	n	%
Sex		
Male	46	55,1
Female	36	43,9
Age (years)		
<40	7	8,53
40 – 49	15	18,28



50 – 59	28	34,15
60 – 69	20	24,4
≥70	12	14,64
Disease time in years (HIV)		
< 5	11	11,86
5 – 10	15	15,26
11 – 15	17	25,42
16 – 20	14	13,56
> 20	25	33,9
ART time in years		
< 5	12	14,63
5 – 10	14	17,07
11 – 15	17	20,74
16 – 20	15	18,29
> 20	24	29,27

Fonte: Soares, L.T.C. *et al.* (2022).

In the analysis between reduction of BMD (osteopenia and osteoporosis) and high cardiovascular risk (high and very high) in 10 years, the statistically significant association was confirmed ($p=0.0120$), with odds ratio demonstrating that there was 3.9 times more chance of a person living with HIV with osteopenia or osteoporosis to present high or very high cardiovascular risk, compared to those with normal BMD (Table 2). In the univariate logistic regression test, patients with osteoporosis had a cardiovascular risk 4.22 times higher than those with normal BMD (95% CI: 1.1-18.63; $p=0.042$).

Table 2 - Association between reduced BMD (osteopenia and osteoporosis) and Cardiovascular Risk (CVR) (high and very high risk) over 10 years in PLHIV under study (Belém-PA, 2022)

	CVR (YES)	CVR (NO)	TOTAL	(p-value)	OR (95% IC)
BMD (YES)	43 (79,6%)	11 (20,4%)	54 (65,8%)	0,0120	3,9091(1,44 -10,55)
BMD (NO)	14 (50%)	14(50%)	28(34,2%)		

Fonte: Soares, L.T.C. *et al.* (2022).



By associating the t-score values for femur and lumbar spine with the percentage of high cardiovascular risk in 10 years, using the Spearman correlation, a statistically significant correlation was found with the femoral t-score ($p=0.0212$), as well as with the t-score for the lumbar spine ($p=0.0159$), which was statistically significant negative (Table 3).

Table 3 - Correlation between T-score of Femoral Neck (FN) and Lumbar Spine (LS) and high CVR in 10 years in the PLHIV under study (Belém-PA, 2022)

		CVR 10 years
T SCORE FN	Spearman (RS)	-0,2540
	p-value	0,0212*
T SCORE LS	Spearman (RS)	-0,2654
	p-value	0,0159*

Fonte: Soares, L.T.C. *et al.* (2022).

Regarding lifestyle habits, the prevalence of smoking was 7.32%. Of the 76 individuals who did not smoke, 31 had a history of previous smoking (40.78%). Few patients (3.66%) had high alcohol consumption (> 3 units of alcoholic beverage/day). The majority (60.98%) did not practice any type of physical activity.

In descending order of prevalence of comorbidities in the sample, lipodystrophy (80.49%) was observed, followed by dyslipidemia (53.66%), arterial hypertension (40.24%) and diabetes (36.59%), an association between high cardiovascular risk and the presence of diabetes ($p= 0.0090$), using the g test for independent samples.

Regarding bone demineralization, most individuals presented reduced BMD (48.78% with osteopenia and 13.41% with osteoporosis). Although there was no statistical association between the reduction in BMD and gender ($p=0.3755$), the prevalence of osteoporosis was higher in females (19.44% versus 8.7% in males). Regarding osteopenia, the opposite was observed: 52.17% were male and 44.44% female (Table 4). There was a statistically significant association between the reduction in BMD and advanced age only in females ($p=0.0084$).



Specifically in relation to HAART, it was found that with longer use of the therapy there was a greater reduction in bone mass ($p=0,0351$). In addition, the drug class used in the regimen was also important: patients using tenofovir (TDF; $p=0.0402$) and protease inhibitors (PI; $p=0.0064$) showed a statistically significant reduction in BMD. However, when the association of HAART and elevated cardiovascular risk was analyzed, it was not statistically significant ($p=0,1487$).

Table 4 - Prevalence of BMD classifications by sex and CVR by sex, in the PLHIV under study (Belém – PA, 2022)

	Male	%	Female	%	p-value (G test)
Bone Mineral Density					0,3755
Adequate	18	39,13	13	36,22	
Osteopenia	24	52,17	16	44,44	
Osteoporosis	4	8,70	7	19,44	
Cardiovascular Risk D: A:D in 10 years					0,0101
Low risk	1	2,22	1	2,78	
Moderate risk	6	13,33	17	47,22	
High risk	16	33,34	6	16,67	
Very high risk	23	51,11	12	33,33	

Fonte: Soares, L.T.C. *et al.* (2022).

The classification of cardiovascular risk using the reduced D:A:D score in 10 years has shown the biggest prevalence of very high cardiovascular risk (42,68%). When categorized by sex (table 4), the classification of very high cardiovascular risk was prevalent in the males (51,11%), while in females the intermediate cardiovascular risk was more prevalent (47,22%). In the G test for independent samples, there was an association between high cardiovascular risk and the male sex ($p=0,0101$).

4. DISCUSSION



In relation to the socio demographic profile of the patients living with HIV, studies showed that men are the primary group affected by the infection in Brazil ¹², a fact that is confirmed by the bigger prevalence of male patients in this sample. About the age range, the group of patients within 50 to 69 years old was predominant, with a time of infection and use of HAART above 20 years. On this basis, a transition of epidemiological scenery is observed, at the expense of the increase in the life expectancy in this portion of the population, provided by the HAART's excellent control over the viral replication^{13,14}.

This study demonstrated that PLHIV with reduced BMD were 3.90 times more likely to develop hard cardiovascular outcomes (cardiovascular death, myocardial infarction, stroke, coronary artery disease), due to CVR (high or very high).

When PLHIV with osteoporosis were evaluated separately, the odds were 4.22 times greater, constituting an independent association between this clinical entity and the increase in CVR, as found in a cohort carried out in a non-PLHIV, in which individuals with osteoporosis had a higher risk of developing coronary artery disease (OR:1.30; 95% CI: 1.23-1.38)¹⁵. Furthermore, there was a statistically significant correlation between the reduction in the T-score of the femoral neck and lumbar spine (the most important variables in determining the diagnosis and prognosis of osteopenia and osteoporosis) with the increase in CVR in this sample.

Several studies reinforce the results found in this research, although they were carried out in people who do not live with HIV. In these, low BMD was both associated with increased severity of arterial calcification¹⁶ and demonstrated independent and incremental value in predicting future atherosclerotic cardiovascular disease ($p < 0.001$)¹⁷.

The relationship between the processes of bone degradation and formation and the pathophysiological changes that lead to the development of cardiovascular diseases is still not fully understood. Despite the common risk factors for changes in bone metabolism and increased cardiovascular risk (inflammation, dyslipidemia, menopause, hypertension, smoking and diabetes mellitus), there is also the possible



contribution of genetics to this bidirectional pathway, in which calcified plaques consist of some bone matrix proteins, such as the matrix GLA protein, bone morphogenetic protein-2, osteoprotegerin and RANKL system, osteocalcin, osteopontin and collagen⁴.

Added to this is the fact that PLHIV in regular use of HAART and in a state of successful viral suppression have an abnormal inflammatory response associated with HIV. Oxidized low-density lipoprotein levels are higher in PLHIV compared to uninfected individuals, which would contribute to both morbidity and cardiovascular mortality and pathological bone loss in this population¹⁸.

About the bone mineral density, it was found a high prevalence in the reduction of BMD in this sample (62,9%), with 3% differences between the sexes, without statistical relevance, deducing that there is a homogeneity in the bone loss by men and women in this study. However, in the stratification of BMD by age, there was a statistical significance only to the female sex, pointing that with a bigger average age there was a bigger reduction in BMD.

In the general population, the men presented a lower risk of osteoporosis before the 70 years of age. But, in men living with HIV, the risk of osteoporosis seems to be bigger and happens earlier, with an increase in BMD reduction after 40 years of age¹⁹. As for women, it is well described in the literature the relation between hypoestrogenism postmenopausal. This is because, besides influencing in an independent way in the reduction of BMD, the infection by HIV can also induce premature menopause²⁰.

In this sample, it was also confirmed what reports in the literature claim: the prolonged time of use and the type of HAART used are significantly associated with the reduction of BMD, especially tenofovir and protease inhibitors. Although the mechanisms that justify this situation are not fully understood, it is known that PLHIV using HAART show considerable changes in biochemical markers of metabolic activity related to the processes of resorption and formation of bone tissue (pyridinoline, alkaline phosphatase, osteocalcin, among others)^{5,19}.



Regarding cardiovascular risk assessment, there is evidence confirming the difference in the prediction of CVR between PLHIV and the general population using traditional risk calculators, such as the Framingham risk score, which underestimates the prevalence of high and very high risk in carriers of the virus. Based on this, the reduced D: A:D model was used in this study, which takes into account the CD4+ count and the type of HAART, to more reliably estimate the CVR of these patients in 10 years²¹.

There was a higher prevalence of very high CVR in this population, with very high CVR in men (51.11%) and moderate CVR in women (47%), with a statistical association between males and very high CVR ($p=0.0101$). Likewise, it was found that the greater the age group, the greater the CVR, with statistical significance both in men ($p=0.0003$) and in women ($p=0.0022$), including males and advanced age in non-modifiable risk factors for the development of CVD, in PLHIV ^{22,23}.

This aging process, otherwise, is associated with the development of multiples comorbidities^{24,25}. The lipodystrophy, an undesirable side effect of the HIV infection and the HAART, was the most prevalent comorbidity in this study (80,49%), followed by dyslipidemia (53,66%), and is intimately associated with the extended period of the disease and drug treatment²⁶.

The lipodystrophy associated with HIV contributes to the morbidity by provoking a redistribution of fat tissue, accumulating (lipo-hypertrophy) or losing fat (lipoatrophy), which lead to the development of insulin resistance, dyslipidemia, visceral fat accumulation and endothelial dysfunction, increasing the risk for cardiovascular disease²⁷. As to the pattern of dyslipidemia in PLHIV, it was observed an association with higher levels of triglycerides and total cholesterol and lower levels of HDL cholesterol, with or without the elevation of LDL cholesterol^{28,29}.

For this reason, the high prevalence of diabetes (36,9%) and pre-diabetes (21,95%) is justified. The prevalence of mellitus diabetes in the general population is around 7,2% and 11,3%, but, in the presence of HIV infection and HAART, it increases to approximately 12%³⁰. The systemic arterial hypertension also presented a high



prevalence in this series (40,24%), compatible with the percentage found in studies conducted in African countries (53%; 35,9%)³¹ and in the Latin-American population, in the sociodemographic and economical aspects as well in the access to healthcare. In conclusion, the aging process and the weight excess are risk factors to has, that has a higher prevalence in PLHIV³².

Based on the physiopathology described and on the comorbidities that can be acquired, the influence of life habits in controlling these risk factors to the development of cardiovascular diseases must be discussed. In the study's sample, 48,10% had a history of past or current smoking. It is known that, in PLHIV, the smoking rate can be 2 or 3 times bigger than in the general population³³.

About the alcohol consumption and sedentarism, a study found a 10,5% rate of alcohol abuse in PLHIV³⁴. Against this backdrop, there was a low prevalence (3,66%) of alcohol abuse in the researched population. Similarly, even though there was an elevated prevalence of sedentarism in this sample, the rate of patients practicing physical activity regularly (39,02%) was superior than the rate described in the general Brazilian population (36,7%)³⁵, which may be a reflection of the regular medical follow-up and the work around health education developed in the endocrinology ambulatory in which these patients are treated.

As limitations of this study, we highlight the relatively small sample size, highly manipulated due to the long period of infection and time of ART use, with an advanced average age, in addition to the fact that some variables associated with cardiovascular risk were not evaluated. Furthermore, the study did not aim to evaluate hormonal, biochemical and drug interference (such as parathyroid hormone levels or the use of polymedicines to treat associated comorbidities), nor were people with previous cardiovascular outcomes excluded from the sample, which naturally increases the prediction of CVR. Despite the limitations, this research draws attention to the relationship between reduced bone mineral density and high CVR in PLHIV, since these comorbidities are more frequent in this population.

5. CONCLUSION



This is a pioneering study that investigated the association between reduced bone density and high cardiovascular risk in the population living with HIV. In this study, common tools that were easy to apply in clinical routine were used (bone densitometry and cardiovascular risk score using an online questionnaire calculator). The conclusion is that individuals with loss of bone mass are 3.90 times more likely to develop cardiovascular events adverse events due to elevated CVR compared to people with normal BMD. Based on this, early screening of cardiovascular risk and changes in bone mass in this population is suggested, in order to diagnose and treat comorbidities with excellence.

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