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**Original Research Article**

# A study of bone mineral density among people living with HIV in India and its correlation with CD4 count

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## ABSTRACT

**Background:** Data on the prevalence of osteoporosis in HIV patients in Asian population is scarce this study was done to find out the prevalence of osteoporosis in HIV infected patients and its correlation with CD4 counts.
**Methods:** This cross- sectional study was conducted in NACO- ART center of tertiary care hospital. Total 115 HIV patients were included in this study which were divided into ART naive (n= 69) and patients taking ART (n= 46). We analysed BMD by DEXA in 115 HIV positive patients and 78 HIV negative age and sex matched controls. Correlation of BMD with a CD4 count and ART regimen was studied.
**Results:** BMD was found to be low in HIV positive patients. T score in HIV positive patients was significantly lower ($p<0.05$) as compared to the HIV negative control group. The prevalence of osteopenia and osteoporosis in HIV positive patients was 50.4% and 29.6% respectively, as compared to 23.1% and 2.6% in HIV negative controls. BMD showed relation with CD4 count. We did not find any statistical difference between any ART regimen and BMD.
**Conclusions:** The prevalence of osteopenia and osteoporosis in HIV infected cases was higher as compared to HIV negative controls and higher in ART group as compared to ART naïve group. Low BMD levels show correlation with low CD4 count. We recommend that HIV positive patients especially with advanced stage of disease, low CD4 count should be screened for low BMD by DEXA scan for osteoporosis and managed accordingly.

**Keywords:** ART, CD4 counts, DEXA, HIV, Osteoporosis

## INTRODUCTION

Human Immunodeficiency Virus (HIV) is a pandemic disease affecting millions of people. The total number of people living with HIV (PLHIV) in India is estimated at 21.17 lakhs (17.11 lakhs-26.49 lakhs) in 2015.[1] HIV infection virtually involves every system of the body, including hematologic, central nervous system, respiratory system, cardiovascular system and skeletal system.

With the advent of Highly Affective Anti-Retroviral Therapy (HAART) there has been a significant increase in the life expectancy of HIV infected patients and as they age newer chronic complications are emerging.[2] Although studies done in the western population have shown increased prevalence of low BMD (bone mineral density) in HIV infected patients in the west but data on the prevalence and associated factors in Asian population is scarce. Therefore, this endeavour was taken with objective to determine prevalence of low BMD in HIV population in northern part of India and study various factors associated with it.

## METHODS

This cross-sectional study was conducted in a tertiary care centre in Northern India over a period of one year.

***Inclusion Criteria***

HIV positive patients visiting HIV clinic or admitted in medicine department (n=115) were included in the study after taking informed consent. The patients were diagnosed as per the criteria specified by National AIDS Control Organisation (NACO), India. Age and sex matched healthy controls (n=78) were also taken for comparison.

***Exclusion criteria***

All subjects with co-morbid conditions which could affect BMD were excluded from the study like neoplastic diseases, rheumatoid arthritis, endocrine disorders, chronic renal disease, and liver disease. Patients on chronic intake of drug that are known to cause osteoporosis like oral corticosteroids, AEDs (ant-epileptic drugs), cyclosporine, and cytotoxic drugs were also excluded from the study. Pregnant females and patients immobilized and bedridden for 6 months or more and those who refused to give consent were also excluded.

***Epidemiological investigations***

Information was recorded in a pre-designed proforma regarding age, sex, smoking habit, body mass index (BMI), co-morbid conditions, past history of fractures, current and previous antiretroviral regimens and cumulative duration of antiretroviral therapy from medical record review. A detailed relevant clinical examination of every enrolled subject was also done.

***Laboratory and radiographic investigations***

Haematological and biochemical parameters were performed at Department of Pathology and Biochemistry of the institute. CD4 count was done using flow cytometry by using Partech-CY Flow Counter Machine Department of Microbiology and BMD was measured by DEXA (dual energy X-ray absorptiometry) scan using the Lunar prodigy advance DEXA system (analysis version 12.30) manufactured by GE Healthcare, installed at Department of Rheumatology. The BMD was measured at 3 sites: Left forearm radius, Left hip and AP spine.

Those with BMD T-score >1 were considered to be normal. Osteopenia was defined as a BMD T-score between -1.0 and -2.5, and osteoporosis was defined as a BMD T-score ≤-2.5 using WHO criteria.[3] During analysis, variables from DEXA scan were treated as ordinal (normal values, osteopenia, or osteoporosis). The statistical analysis was done using SPSS (Statistical Package for Social Sciences) version 16.0 statistical analysis software.

All subjects who were diagnosed to have low BMD values as suggested by low T scores and low Z scores were referred to a specialist for further work up and appropriate management.

***Definitions***

HAART (Highly active anti-retroviral therapy) was defined as a combination of at least 3 anti-retroviral agents that contained 2 nucleoside reverse transcriptase inhibitors (NRTI) plus 1 protease inhibitor (PI) or 1 Non-nucleoside reverse transcriptase inhibitor (NNRTI). None of our subjects was on Protease Inhibitor (PIs) containing regimen. Being underweight was defined as having BMI <18.5kg/$m^2$ based on the WHO standards for Asian population.

## RESULTS

The mean age of HIV positive group was 36.08±6.74years and the mean age of HIV negative control was 36.50±6.87 years (p>0.5). It was important to have age matched comparison groups because it is a well proven fact as age advances bone mass loss occurs.

**Table 1: Study population characteristics.**

| Variable | Cases | Naïve | ART | HIV negative controls |
|---|---|---|---|---|
| Number (n) | 115 | 69 | 46 | 78 |
| Mean Age (years) | 36.08±6.74 | 36.17±7.17 | 35.93±6.11 | 36.50±6.87 |
| Male (n) | 75 (65.2%) | 45(65.2%) | 30(65.2%) | 51(65.4%) |
| Female(n) | 40(34.8%) | 24(34.8%) | 16(34.8%) | 27(34.6%) |
| BMI(kg/m2) | 19.97±2.17 | 20.55±2.36 | 19.09±1.48 | 23.35±2.18 |
| History of Fracture | No | No | No | No |
| Mean CD4 count | 280.40±112.73 | 316.23±137.58 | 227.49±187.25 | NA |
| Mean Hemoglobin (g/dl) | 10.18±1.2 | 10.1±1.2 | 10.3 ±0.9 | 13.6±1.4 |
| TSH (µ IU/ml) | 2.096 | 2.08 | 2.12 | 2.1 |
| Serum Phosphorous | 2.94 | 2.99 | 2.87 | 3.02 |
| Serum Calcium (mg/dl) | 8.6 | 8.7 | 8.4 | 8.8 |
| Serum Albumin (mg/dl) | 3.16 | 3.2 | 3.1 | 4.3 |

**Table 2: Prevalence of low bone mass in cases and controls.**

| BMD STATUS | Cases (n=115) | | Control (n=78) | |
|---|---|---|---|---|
| | No. | % | No. | % |
| Normal | 23 | 20.0 | 58 | 74.4 |
| Osteopenia | 58 | 50.4 | 18 | 23.1 |
| Osteoporosis | 34 | 29.6 | 2 | 2.6 |

p <0.001

The gender distribution amongst cases and control group was also similar (Table 1). Among HIV positive cases males were 65.2% (n=75) and among HIV negative controls male were 65.4% (n=51) (Table 1). Among HIV positive cases females were 34.8% (n=40) while in the control group females were 34.6% (n=27) This was important because it is a well proven fact that females have lower bone mass and have a faster rate of decline too as compared to males.[3]

The results showed a significant difference in percentage of osteopenia and osteoporosis among HIV positive and normal healthy controls (Table 2). In the cases 50.4% (n=58) had osteopenia as compared to 23.1% in the control group (n=18). Osteoporosis was seen in 34 cases (29.6%) as compared to only 2 cases in controls (2.6%). Normal BMD was seen in only 20% of cases as compared to 74% in controls. Hence we found that osteopenia and osteoporosis was more in HIV positive population as compared to HIV negative controls and this difference was statistically significant (p<0.001).

We measured BMD at 3 sites (left forearm radius, AP spine, left neck femur) and found that cases had lower BMD, T scores and Z score as compared to HIV negative controls and this difference was statistically significant for all the parameters measured (Table 3). Lower Z scores in the cases as compared to the controls also shows that HIV population has bone loss over and above caused by ageing or female sex alone.[4-6]

**Table 3: BMD and T-Score comparison between cases and controls.**

| Variable | Cases (N=115) | | Controls (N=78) | | Significance Of Difference (Cases V/S Control) | |
|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | t | p value |
| **BMD** | | | | | | |
| AP Spine | 0.91 | 0.14 | 1.15 | 0.17 | -10.53 | <0.001 |
| Left Neck Femur | 0.85 | 0.18 | 1.09 | 0.10 | -8.70 | <0.001 |
| Left Forearm Radius | 0.84 | 0.12 | 1.02 | 0.11 | -7.19 | <0.001 |
| **T-Score** | | | | | | |
| AP Spine | -1.71 | 1.20 | -0.38 | 1.44 | -7.01 | <0.001 |
| Left Neck Femur | -1.74 | 0.94 | -0.59 | 0.75 | -8.97 | <0.001 |
| Left Forearm Radius | -1.55 | 0.99 | -0.41 | 0.91 | -8.18 | <0.001 |
| Z-Score | -1.33 | 1.01 | -0.22 | 1.41 | -6.35 | <0.001 |

**Table 4: Co- relation of BMD with CD4 counts.**

| CD 4 count | | cases<250 | | >250 | | P Value |
|---|---|---|---|---|---|---|
| | BMD | NO. | % | NO. | % | |
| Cases | Normal BMD | 4 | 5.5 | 19 | 45.2 | p<0.001 |
| | Osteopenia | 41 | 56.2 | 17 | 40.5 | |
| | Osteoporosis | 28 | 38.4 | 6 | 14.3 | |
| ART Naive | Normal BMD | 2 | 4.5 | 10 | 40.0 | p=0.001 |
| | Osteopenia | 31 | 70.5 | 13 | 52.0 | |
| | Osteoporosis | 11 | 25.0 | 2 | 8.0 | |
| ART group | Normal BMD | 2 | 6.9 | 9 | 52.9 | p=0.002 |
| | Osteopenia | 10 | 34.5 | 4 | 23.5 | |
| | Osteoporosis | 17 | 58.6 | 4 | 23.5 | |

The cases were further divided into two groups depending on the CD4 counts with Group A cases having ≤250/µl and Group B having CD4 >250/µl. In Group A, osteopenia was present in 56.2% and osteoporosis was found in 38.4% as compared to 40.5 % and 14.3% respectively in Group B (Table 4). Normal BMD was present in 45.2% in Group B as compared to 5.5% in Group A. This difference in low bone mass (osteopenia and osteoporosis) between HIV positive cases having CD4 counts ≤250/µl and >250/µl was statistically significant (p<0.001).

Out of 46 patients on ART, 21 patients were taking ZLN (zidovudine, lamivudine and nevirapine) 9 were on ZLE (zidovudine, lamivudine and efavirenz), 8 were taking TLN (tenofovir, lamivudine and nevirapine), 6 patients were on TLE (tenofovir, lamivudine and efavirenz) and 2 were on SLE (stavudine, lamivudine and efavirenz). Majority of the patients were in the ZLN group and none of the subjects were on protease inhibitor. We did not find any statistical difference between any ART regimen and BMD (Table 5).

**Table 5: Correlation between ART regimen and BMD Grading.**

| BMD | ART Regimen | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | ZLN | % | ZLE | % | TLN | % | TLE | % | SLE | % |
| Normal | 5 | 23.81 | 3 | 33.33 | 2 | 25 | 1 | 16.67 | 0 | 0 |
| Osteopenia | 6 | 28.57 | 2 | 22.22 | 2 | 25 | 3 | 50 | 1 | 50 |
| Osteoporosis | 10 | 47.62 | 4 | 44.44 | 4 | 50 | 2 | 33.33 | 1 | 50 |
| Total | 21 | | 9 | | 8 | | 6 | | 2 | |

$\chi^2$=2.52 (df=8); p=0.961; E=Efavirenz,L=lamivudine, N=Nevirapine, T=Tenofovir, Z=Zidovudine, S=Stavudine.

## DISCUSSION

The factors influencing bone mass are race and area dependent. Studies conducted in western countries or other parts of this country may not reflect directly on the bone health in a given region. Multiple studies have shown increased prevalence of low bone mass (osteopenia and osteoporosis) in HIV positive patients but studies from India are scarce therefore we took up this endeavour to study bone mineral density in HIV positive population from our area and if found low, its correlation with various factors both HIV related (CD4 count) and HIV independent factors (BMI).

Among HIV-infected persons, the aetiology of osteoporosis is multi-factorial. Traditional risk factors such as smoking, alcohol use, physical inactivity, low body weight, vitamin D deficiency, hypogonadism and opiate use contribute to the increased risk. Apart from these, direct effects of antiretroviral therapy (ART) and chronic immune activation by HIV infection also play an important role.[7-9] Typically, bone remodelling involves the tightly coupled processes of bone resorption and bone formation. In untreated HIV, through direct viral effects and inflammatory effects, bone resorption and bone formation are uncoupled.[10] In vitro studies have shown that HIV viral proteins Vpr and gp120 stimulate osteoclast activity and p55-gag suppresses osteoblast activity and increases osteoblast apoptosis.[11-13]

In addition, inflammatory cytokines, such as tumour necrosis factor α and interleukin 6 promote osteoclasto-genesis and bone resorption.[14-16] High concentrations of HIV RNA have been associated with elevated levels of receptor activator of nuclear factor κ β ligand (RANKL), an osteoblast secreted cytokine that promotes osteoclast formation.[17]

Osteoporosis was reported in about 10-13% of HIV subjects in western population.[18] In present study osteoporosis was percent in 29.6% of cases which is much higher as compared to study by Bruera et al.[18] There could be several reasons. Indian HIV population lives in a resource limited setting and a great proportion of them are diagnosed in an advanced stage of disease with frequent OIs (opportunistic infections).[19] Other factors such as malnutrition and Vitamin D deficiency might also be causing these increased prevalence of osteoporosis.[20]

Casado et al studied 285 subjects and found the prevalence of osteopenia in HIV population to the tune of 36% to 57% depending on the presence of secondary factors.[19] Present present study also found osteopenia in 50.4% of cases which is in the same range. Knobel et al found osteopenia and osteoporosis both in patients on HAART treatment and in therapy-naive patients.[21] The HIV-patients group showed significant differences from the non-HIV, healthy control group but there was no difference in prevalence of osteopenia and osteoporosis in HIV-infected individuals, both on therapy and therapy-naive.

Without doubt present study shows that HIV patients have significantly higher percentage of osteoporosis as compare to healthy controls of same age. Moreover in our study the mean age of HIV positive cases having osteoporosis was 33.2 years while the mean age of the control having osteoporosis was 47.5 years (p<0.05). Thus we can infer that the HIV positive patients loose

bone mass earlier as compared to general population. The relation of CD4 count and low bone mass in HIV was studied by Grant PM et al.[22] In multivariable analysis, baseline CD4 cell count was significantly associated with 96-week BMD loss; individuals with baseline CD4<50 cells/µL lost significantly more bone loss compared to those with CD4≥500 cells/µL. Other factors like older age, female sex, lower BMI, higher HIV-1 RNA levels, and PI and tenofovir assignment were also associated with greater BMD decline. To study relationship between CD4 count and bone loss we took cutoff of 250 cells per cubic litre. In India HARRT was initiated if CD4 counts were below 250 cells per cubic litre or WHO stage III and IV as per NACO guidelines. Recently these have been changed and recent cutoff is 350 cells per cubic litre. Present study shows that difference in low bone mass (osteopenia and osteoporosis) between HIV positive cases having CD4 counts ≤250/µl and >250/µl was statistically significant (p<0.001).

In the present study we also found that higher percentage of low BMI in HIV positive subjects was associated with low BMD. This finding was also reported by Bolland MJ et al that low body weight in HIV infection was associated with low BMD.[23] At all skeletal sites BMD was lower by 4.4-7.0% in the HIV-infected groups than the controls (P<0.01). After adjustment for body weight, residual between-groups differences in BMD were small (2.2-4.7).

***Limitations of the study***

In the present study there are some limitations. This study was done in a single centre and number of subjects studied is small and this is an observational study and not a prospective one, hence it cannot establish causal relationship of HIV with low bone mineral density. None of our HIV patient was on protease inhibitor (PI) as according to NACO guidelines, PI group is preserved as second line drugs to be given in resistant cases. Base line BMD of HIV positive patients prior to initiation of HAART was not available so low BMD in those on ART cannot be attributed to HAART alone.

## CONCLUSION

To conclude low bone mass is more prevalent in HIV positive population as compared to HIV negative healthy population. Bone loss in HIV positive individuals occurs at an earlier age as compared to HIV negative controls. High prevalence of osteopenia and osteoporosis in HIV positive population is associated with multiple factors. Low body mass index (BMI), advanced stage of disease, low CD4 counts are associated with low bone mass in HIV positive individuals. Further studies with large sample size are needed to identify and evaluate the magnitude of low BMD problem in Indian HIV population and to demonstrate independent effect of each variable on bone mass. We recommend that screening for low bone mass by DEXA scan and vitamin D levels in HIV positive population should be done periodically and those found to have low bone mass or vitamin D deficiency should be referred to a specialist for appropriate therapy.

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