ORIGINAL ARTICLE

Bone health parameters in HIV positive patients not receiving antiretroviral therapy - An observational cross-sectional study in India

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Our primary aim was to assess point prevalence of osteoporosis/ osteopenia and overall BMD status based on dual energy X-ray absorptiometry (DXA) scan. Our secondary objectives were to correlate BMD with serum Vitamin D levels and to identify possible risk factors for osteoporosis and osteopenia in this population.

Key Words: *BMD; HIV; Menopausal; Parathormone; Osteopenia*

INTRODUCTION

Low bone mineral density (BMD) has been observed in HIV patients on antiretroviral therapy (ART) [1]. They have 4.3 times higher fracture rates [2,3] which further increase over years of follow-up. HIV patients on ART have 6.4-fold increased risk of osteopenia and 3.7-fold increased risk of osteoporosis compared to HIV-uninfected controls [4]. There is limited data on prevalence of osteopenia or osteoporosis in ART-naïve HIV positive patients. Our study was undertaken to estimate prevalence of low BMD in ART naïve HIV patients.

METHODS

This cross-sectional observational study was carried out at a tertiary care hospital in western Maharashtra, India between October 2014 and Oct 2016 in subjects above 18 years, being HIV positive for at least 03 years and not on ART or any calcium or vitamin D supplements. We excluded patients with pregnancy and other conditions predisposing to osteopenia (such as chronic kidney disease, prolonged glucocorticoid use, hyperthyroidism, malignancy, chronic obstructive airway diseases, immunosuppressant therapy, hypogonadism, anti-convulsant medication, hyperparathyroidism, prolonged immobilization, rheumatoid arthritis, and ankylosing spondylitis). Critically ill and bed-ridden patients were also excluded. A sample size of 100 was derived by reported prevalence of osteoporosis in HIV-infected cohorts as 15% [5]. We clinically assessed 108 patients and measured their serum albumin, calcium, phosphate, urea, creatinine, alkaline phosphatase (ALP), 25-hydroxy-vitamin-D, parathormone (iPTH), and urinary calcium-creatinine ratio, from a National accreditation board for testing and calibration of laboratories (NABL) and ISO 151819:2007 accredited laboratory. DXA scan of three sites viz. right forearm, right femur and lumbosacral (LS) spine was done by Hologic Discovery Model which utilizes one pass single-sweep scanning and a multi-element digital detector array paired with true fan-beam acquisition geometry, enabling rapid, dual-energy BMD measurements. The BMD was assessed in terms of WHO assigned T&Z scores. The BMD measured at femur and/or LS was taken into account for assessment of BMD.

STATISTICAL ANALYSIS

We used Chi-square test, Fishers' exact test and multivariate analysis for analysis of data using IBM SPSS 2015 version 22.0.0.

ETHICAL CONSIDERATIONS

Written consent in Marathi/Hindi & English was taken from each patient and their identity was kept confidential. Each patient was identified by a serial number written on the protocol form. Approval from state and national level authority was taken.

RESULTS

The mean age of the study population was 37 years (37 ± 12.25, 20-59 years). Other baseline data are depicted in Table 1. A total of 89 (82.4%) patients had low vitamin D and among them 15 (16.85%) had osteoporosis and 32 (36%) had osteopenia. However, patients with normal vitamin D levels did not show low BMD. In this study we found that low BMD was significantly associated with increasing age, duration of HIV, low BMI, low serum albumin, calcium, vitamin D and low baseline CD4 levels.

DISCUSSION

The higher prevalence of low BMD and OP in People living with HIV/AIDS (PLHA) is a well-known fact. However, most of these prevalence studies are

TABLE 1

Baseline parameters and variations in bone mineral density

Parameters	Mean ± SD (Range)	Osteoporosis Number (%)	Low BMD Number (%)	P-value
Age	37 ± 12.25 (20-59)			
Below 40 years	43 (39.8)	0(0)	10 (15.38)	0.001
Above 40 years	65 (60.18)	15 (34.8)	37 (86)	
Sex				
Male	No (%)-63 (58.33)	8 (12.6)	31 (49.2)	0.078
Female	No (%)-45 (41.67)	7(15.5)	16 (35.5)	
Menopausal status				
Post-menopausal	No (%)-17(15.7)	7(41.1)	13(76.47)	
Others	No (%)-91(84.3)	8 (8.7)	34 (37.36)	
BMI	21.5 ± 2.52(16.8-28.1)			
Low	9 (13.8)	7 (77.7)	8 (88.8)	0.001
Normal	99 (91.66)	8(8.1)	39 (39.3)	
Base line CD4	578.06 ± 130.58 (380-790)			
Above 500 cells/mcl	68 (62.96)	0	9 (13.2)	0.001
Below 500 cells/mcl	40 (37.03)	15 (37.5)	38 (95)	
Duration of HIV (in years)	5.31 ± 1.71(3-8)			
>5	52 (48.2)	15 (28.8)	39 (75)	0.001
<5	56 (51.8)	0	8 (14.2)	
Sun light Exposure (in hours)	4.25 ± 1.46 (2-6)			
>4	55 (50.92)	0	8 (14.5)	0.001
<4	53 (49.07)	15 (28.3)	39 (73.5)	

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from Western countries. There is very little data in this regard from India. Most of the studies have not clearly mentioned whether their subjects were ART naive or on therapy.

The osteoclastic bone resorption is favored by the increased osteoclast precursors in peripheral blood and imbalance in the RANKL/OPG ratio [6]. Despite effective treatment, an inflammatory undercurrent persists in these patients [7,8]. Opportunistic infections and lipopolysaccharides induced inflammation stimulate increased secretion of IL-1, TNF and RANKL [9]. These, in turn cause increased bone resorption. Various lifestyle factors like physical inactivity, smoking, alcohol consumption, opiate abuse, depression, reduced oral intake of calcium and vitamin D, malabsorption and low testosterone levels are responsible for low BMD in PLHA [10]. Low BMI in PLHA is also associated with low BMD [11]. In some studies the use of protease inhibitors and TDF was associated with an increased risk of osteopenia and OP [4,12,13] but similar other studies failed to confirm this association [11,14].

A meta-analysis of pooled data from 11 cross-sectional studies between 2000 and 2005 by Brown et al. along with a review of literature from different studies from 1993 to 2005 demonstrated a prevalence of OP & reduced BMD to be 15% & 67% (40% to 87.5%) respectively in 884 HIV-positive individuals compared to 654 HIV-negative age and sex-matched controls. The unadjusted odds for OP were 3.7 times more in PLHA as compared to uninfected controls [4]. Several studies have reported a higher prevalence of bone fractures in PLHA and have linked it to the age of the patients [15-19]. A Spanish observational study looked at BMD in young adults between 20 - 30 years. They compared bone health of 232 HIV-positive young adults with age and sex-matched 75 HIV-negative controls. The prevalence of osteoporosis was 11% in PLHA & 4% in controls. Osteopenia was found in 57% of PLHA and in 51% of the controls, suggesting an earlier onset of osteoporosis in PLHA as compared to HIV-negative people [20]. In our study, point prevalence of OP is 13.88% in a population which has mean age of 37 years. This hints towards the fact that HIV infection per se is an independent risk factor for low BMD.

The prevalence of low BMD & OP is more in women with HIV compared to non-infected controls. The comparative data on ART naïve and on-ART women is not known [21]. One large study on 'HIV and bone health' reported that women had significantly lower BMD at lumbar spine and total hip (lower Z-scores) than men [21]. Our study demonstrated osteoporosis in 15.56% of female patients and 12.7% of male patients. However, overall low BMD was observed in 49.2% male and 35.5% female population. This may be due to relatively low proportion of postmenopausal women in this study and the number of men above 50 years was more than number of women in the same age group (Table 2).

When compared to two other Indian studies, the prevalence of osteoporosis was higher among HIV positive women (9% in pre-menopausal & 41% in post-menopausal) as compared to HIV negative women (7.6% in pre-menopausal & 25.8% in post-menopausal). This, again, points to an association between HIV infection and low BMD. As per one study, the overall prevalence of osteoporosis and osteopenia in men above 50 years was approximately 8.5% and 42% respectively [22]. Considering the mean age of 37 yrs in our study, the prevalence of OP in male patents (12.6%) and overall low BMD (49.2%) was significantly high (Table 3).

A baseline CD4 count of <500 cells/ml has been associated with worse prognosis in fragility fractures attributed by a persistent pro-inflammatory state [23]. Multivariate analysis, from our study, keeping BMD as dependent variable, showed correlation of low BMD with low baseline CD4.

As per bone mineral density sub-study of START trial, duration of HIV infection has a correlation with reduced BMD compared to general population [24-26]. Our study also revealed that duration of HIV infection had significant correlation with low BMD.

In a study Vitamin D levels were estimated in 96 individuals; (with 82% males & 18% females & median age of 40.1 years) significantly low levels of vitamin D were found in women with veiled dressing style [27]. Whether HIV patients require more sunlight exposure to synthesize enough vitamin D is not documented in literatures. However, in our study, we found significant association between low BMD and low sunlight exposure.

In an Indian study from rural population, 65.4% of non-HIV patients attending medical OPD had low BMD [28]. In our study 82.4% patients had low vitamin D level; among them 16.85% had osteoporosis, 35.96% osteopenia and 52.81% patients had low BMD. This suggests that PLHA have higher prevalence of hypovitaminosis D and also suggests that low vitamin D level is associated with low BMD (p value <0.001).

The mean plasma albumin concentration was found to be lower in HIV infected individuals whether they were on ART or not compared to healthy controls [29]. Hypoalbuminemia can promote osteoclastic activity via RANKL and via associated inflammatory cytokinemia [30,31]. Other

TABLE 2

BMD final outcome data

BMD sites	Score	Mean ± SD (Range)	Osteoporosis Number (%)	Osteopenia Number (%)	Low BMD Number (%)	Normal BMD Number (%)
Right Forearm	T	-0.311 ± 1.56 (-2.97-2.95)	17(15.74)	23 (21.29)	40 (37.04)	68 (62.96)
	Z	-0.53 ± 1.67 (-2.96-2.83)	17(15.74)	27 (25)	44 (40.74)	64 (59.26)
LS Spine	T	-0.3 9 ± 1.68 (-2.9-2.99)	15 (13.88)	28 (25.92)	43 (39.81)	65 (60.19)
	Z	-0.42 ± 1.69 (-2.96-2.87)	15 (13.88)	25 (23.14)	40 (37.04)	68 (62.96)
Right Femur	T	-0.47 ± 1.61 (-3-2.98)	15 (13.88)	26 (24.07)	41 (37.96)	67(62.04)
	Z	-0.52 ± 1.57 (-2.99-2.69)	15 (13.88)	32 (29.62)	47 (43.52)	61 (56.48)

Table 3

Multivariate analysis placing BMD as dependent variables

Parameters	Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95.0% Confidence Interval for B	
	B	Std. Error	Beta			Lower Bound	Upper Bound
Age	.039	.034	.281	1.155	.251	-.028	.106
BMI	-.028	.076	-.043	-.374	.709	-.179	.122
base line CD4	.015	.004	1.114	4.050	.000	.007	.022
Calcium (mg/dL)	.787	.367	.229	2.142	.035	.058	1.516
Serum albumin (g/dL)	-1.406	.450	-.407	-3.127	.002	-2.298	-.514
ALP (IU/L)	-.001	.008	-.006	-.106	.916	-.017	.015
iPTH (ng/dL)	-.015	.007	-.213	-2.150	.034	-.030	-.001
25 (OH) Vitamin D (ng/ml)	-.005	.015	-.027	-.367	.714	-.035	.024

probable mechanisms may be the association of hypoablbuminemia with poor nutrition, immobility [32] reduced transport of minerals which finally form calcium phosphate apatite crystals, decreased Gla-protein, increased parathormone (PTH) and decreased vitamin D binding protein [33-36]. Our study found and association of hypoalbuminemia with low BMD.

Studies have shown that HIV infection per se and ART both can cause greater tendency to OP, secondary to reduction of calcium & vitamin D and the increase of levels of parathyroid hormone (PTH) [37]. The increase in PTH is secondary hyperparathyroidism (SH). In our study statistically significant correlation was observed between increased level of iPTH and low BMD.

Low serum calcium levels have been shown to correlate with low BMD (p value <0.001). Though the subjects screened in our study had normal urine calcium/creatinine ratios and none of them had chronic renal disease or had been taking calcium supplementations, low calcium levels were seen mainly in those who had low vitamin D levels and high iPTH levels which can be interpreted as effects of HIV on bone demineralization and increased resorption and decreased anabolism.

The role of hormones LH, FSH, prolactin or testosterone in reducing BMD especially in ART naïve HIV patients has not been very clear as far as different studies around the world are concerned [10,38,39]. In our study, no statistically significant correlation was observed between low BMD and FSH, LH & prolactin levels. However, males with low testosterone had significant low BMD which is in keeping with previous study findings [40]. Osteoclast differentiation and bone resorption is mediated by direct inhibitory effect of testosterone but not estrogen (E2). E2 activation causes inhibition of osteoclasts indirectly via osteoblasts [41]. Moreover, ERa on osteoclasts seems to regulate bone resorption in women but not in men, whereas bone resorption in men is partially mediated by AR signaling on osteoblasts [42]. HIV infected men are more likely to develop androgen deficiency than in the general population.

Our study is unique in the sense that it clearly demonstrates a high prevalence of low BMD in ART naïve patients. The study suggests that HIV per se is a risk factor for low BMD (OP & osteopenia). The prevalence of low BMD in PLWH in India is significantly high and needs urgent attention. All PLHA in India above 50 years should be screened for low BMD to prevent further complications. Controversy exists about the role of screening for vitamin D deficiency in HIV patients. The Institute of Medicine, a nonprofit organization established in 1970 as a component of the US National Academy of Sciences did not recommend screening. European AIDS Clinical Society (EACS) however recommends screening for vitamin D deficiency at the time of diagnosis of HIV and thereafter every 2-year [43]. The Endocrine Society recommends screening in patients who have high risk for deficiency like patients on ART, advanced age, postmenopausal state and history of pathological fractures [44]. There is no such recommendation in India. HIV positive patients in India should be investigated for Vitamin D deficiency, hypoalbuminemia, secondary hyperparathyroidism, androgen deficiency and low serum calcium. Timely interventions can defer OP related complications and improve quality of life.

Since, it was an observational, cross-sectional study we did not include controls. The results were not compared with non-HIV & on ART populations from other Indian and international studies. Due to resource limited setting, HIV viral load was not assessed. The DXA scan readings at different sites were not compared and any T or Z value falling in the osteopenic or osteoporotic range was taken as positive. The absolute precision of the study was taken as 7% to optimize sample size as per budget.

Recent CDC guidelines on initiation of ART has included all patients irrespective of CD4 cell counts which is expected to have deep impact on the transmission and prevention of the disease along with treatment of the host. Life expectancy of a person living with HIV will increase and so will the age-related morbidities and non-communicable diseases like low BMD, osteoporosis and related issues. HIV per se is a risk factor which can cause concurrent low calcium, vitamin D deficiency, albumin deficiency and increased parathormone levels leading to more bone resorption. The possible risk factors which accelerate the process are advanced age, female sex, menopausal status, low BMI, increased duration of the disease, less sunlight exposure and low baseline CD4 counts. As per the 2014 'National Osteoporosis Foundation Clinicians Guide to the Prevention and Treatment of Osteoporosis, USA' recommendation, screening of osteoporosis is indicated in women of age 65 years or older and men of age 70 years and older (regardless of clinical risk factors), peri/postmenopausal women, and men in the age group of 50–69 years with clinical risk factors for fracture [45]. Our study has showed that HIV infection per se is an important risk factor for low BMD (osteoporosis) which is matching with the result of various other relevant studies.

CONCLUSION

HIV infection per se is a risk factor for low BMD and associated complications. The risk factors are increased age, low baseline CD4 level, duration of the disease, low sunlight exposure, low BMI, low serum levels of vitamin D calcium, albumin and testosterone. Therefore, among HIV patients with the above risk factors, early screening for osteoporosis will be beneficial and avert many complications related to osteoporosis by timely interventions.

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