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Original Research Article

Study of Bone Mineral Density in HIV Infected Patients and its Correlation with Different Anti Retroviral Regimes

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Abstract:

Introduction: The advancement in the understanding of Human Immunodeficiency Virus (HIV) infection, the improved prophylaxis, treatment of opportunistic infections and the combination Anti-retroviral therapy (cART) has greatly prolonged the survival of HIV patients. Human immunodeficiency virus (HIV) specifically, has a negative impact on BMD and although highly active antiretroviral therapy increases the prognosis for HIV-infected individuals, BMD still seem to decrease further.

Objective: To evaluate the Bone Mineral Density in HIV Infected Patients and Its Correlation with Different Anti-Retroviral Regimes.

Materials & Methods: The study was conducted in Department of Medicine at MDM Hospital and the ART centre, both attached to Dr. S.N. Medical College, Jodhpur. Study included both patients who are under cART regime or who are treatment naïve, patients were selected randomly both out-patient and in-hospital based. DEXA scan of lumbar spine was done using Hologic Discovery Wi (S/N 86537) model.

Results: Osteopenia was found in 38(44.18%) patients, osteoporosis in 16(18.6%) patients and 32(37%) were having normal BMD which suggest that there is a high prevalence of low BMD in HIV infected patients. The mean nadir CD4 count among the study subjects was 244.59±162.62. Patients with low BMD (both osteopenia and osteoporosis combined) had mean nadir CD4 count low(215.98±141.51) compared to nadir CD4 count of patients with normal BMD (292.82±185.59) which was statistically significant($p<0.05$months for AZT based regime and 17.68±18.83 months for TDF based regime.

Keywords: BMD, CD4, cART.

Introduction

The advancement in the understanding of Human Immunodeficiency Virus (HIV) infection, the improved prophylaxis, treatment of opportunistic infections and the combination Anti-retroviral therapy (cART) has greatly prolonged the survival of HIV patients. Now interests arise in the long term complications of HIV and its ART, as HIV infected patients now have increased lifespan compared to the pre-ART era. In the post-ART era, opportunistic infections are replaced by the long term complications of HIV infection itself and of cART. [1] Long term cART is found to be associated with several metabolic and morphological complications including lipodystrophy, insulin resistance, diabetes, dyslipidemia, osteopenia and osteoporosis. [2-4] The prevalence of bone abnormalities in HIV-infected patients is several fold higher than that in age, race and sex matched control groups. The etiology of low bone mineral density (BMD) in HIV infected patients seems to be multifactorial Risk factors include low body mass index (BMI), tobacco consumption, alcohol consumption, concurrent tubercular disease, steroid therapy, antiepileptic drugs, physical inactivity, low calcium and vit D intake in diet, old age, HIV infection itself and cART drugs. [5-9] Furthermore, indirect HIV/AIDS-associated pathologies such as muscular degeneration, kidney disease, and imbalance in sex steroids and calcitropic hormones such as parathyroid hormones (PTH) and vitamin D, likely also contribute to low bone mass at some level with different combination of these factors affecting bone mass in different patients. [10] The role of immune system and disruptions to the immune-skeletal interface in the bone loss associated with HIV-infection has only recently been explored. Because the production and regulation of osteoclastogenic cytokines such as Receptor activator of nuclear factor kappa-B

ligand (RANKL) and *osteoprotegerin (*OPG) are under influence of key cells of immune system that are specifically targeted by HIV infection (T-cells), and cells regulated by T-cells (B-cells and monocytes), the skeletal abnormalities encountered in HIV infection may be mediated via this

deregulation of immune system. Protease inhibitors (PI) are potent inhibitors of cytochrome CYP3A4, which inhibits 1-hydroxylase enzyme. [11] Even though the exact mechanism of bone loss due to PIs is unknown, it is hypothesized that PIs induced accumulation of prelamin-A, a biomarker of cell aging leading to cell senescence which could induce premature aging of osteoblast precursors, human marrow mesenchymal cells and affect their differentiation to osteoblast [12]. PIs increase osteoclast differentiation and alter vit D metabolism [13]. Nucleoside reverse transcriptase inhibitors (NRTIs) inhibit the enzyme DNA polymerase leading to loss of structure and function of mitochondrial DNA thereby causing cellular oxidative stress [14-15]. Tenofovir damages the proximal tubules of kidney causing phosphate wasting which impedes bone mineralization. Zidovudine (AZT) promotes dose dependent increase in the activity of Tartrate-Resistant Acid Phosphatase (TRAP) promoter and the nuclear factor kappa B (NFkB) transcription factor promoting osteoclastogenesis and thereby increasing bone resorption [16]. Efavirenz is associated with low vit D levels[17] the effect is hypothesized to occur through induction of 24-hydroxylase, a cytP450 enzyme that inactivates 25-OHD and 1, 25-OHD. [17-21] It is probable that all the anti-retroviral drugs are detrimental to the skeleton. [22]

Material and Methods:-

The study was conducted in Department of Medicine at MDM Hospital and the ART centre, both attached to Dr. S.N. Medical College, Jodhpur. Participants after understanding the study protocol and procedures were asked to given their written consents for the study. The study is a hospital based cross-sectional study where the study populations comprise of HIV infected patients of western Rajasthan who attended the ART centre attached to Dr SN Medical College, Jodhpur. Study included both patients who are under cART regime or who are treatment naïve, patients were selected randomly both out-patient and in-hospital based, who were admitted in MDM Hospital attached to Dr SN Medical College, admitted for concomitant or other unrelated illness.

Inclusion Criteria:

A. HIV infected patients who are registered in ART centre of Jodhpur and neighboring district of western Rajasthan.
B. Treatment naïve patients.
C. Treatment with combination ART for at least 2 months
D. Age of patients was between 20-55 years.

Exclusion Criteria:

A. More than 55 years and less than 20 years of age.
B. Pregnant or post-menopausal women.
C. Patients who were diagnosed or were under treatment for chronic liver, kidney, thyroid or parathyroid dysfunction or failure and any known malignancy.
D. Who were chronic alcoholic, chronic smoker or are on long term drug therapy which could adversely affect the bone metabolism.
E. Any known history of fragility fractures or treatment for osteoporosis.

Total 86 patients included in the study among which 17 patients are treatment naïve and 69 patients on cART regime. Patients on cART regime are divided into two groups i.e. 25 patients on Zidovudine (AZT) based regime and 44 patients on Tenofovir (TDF) based regime. Comprehensive data was taken which included name, age, sex, occupation, smoking habits, alcohol intake, dietary habits, height, weight, BMI, known duration of HIV infection, nadir and present CD4 counts, types and duration of antiretroviral therapy. Laboratory investigation including complete blood counts, blood sugar, renal function tests, liver function tests, serum level of calcium, phosphorus, alkaline phosphatase, serum vit. D level and thyroid profile were done to rule out other chronic liver, renal, thyroid diseases or diabetes mellitus.

DEXA scan of lumbar spine was done using Hologic Discovery Wi (S/N 86537) model in MDM Hospital, Jodhpur. Serum Vit D level was accessed in 35 patients who were randomly selected, regardless of their treatment status, 25-hydroxyvitamin D- ELISA, Chemiluminiscence Macro particle Enzyme Immunoassay (CMIA) method was used for analysis of vit D level. Patients were classified according to WHO criteria as having-Normal BMD – T-score -1.0 and above

Osteopenia – T-score between -1.0 and -2.5

Osteoporosis – T-score -2.5 and below

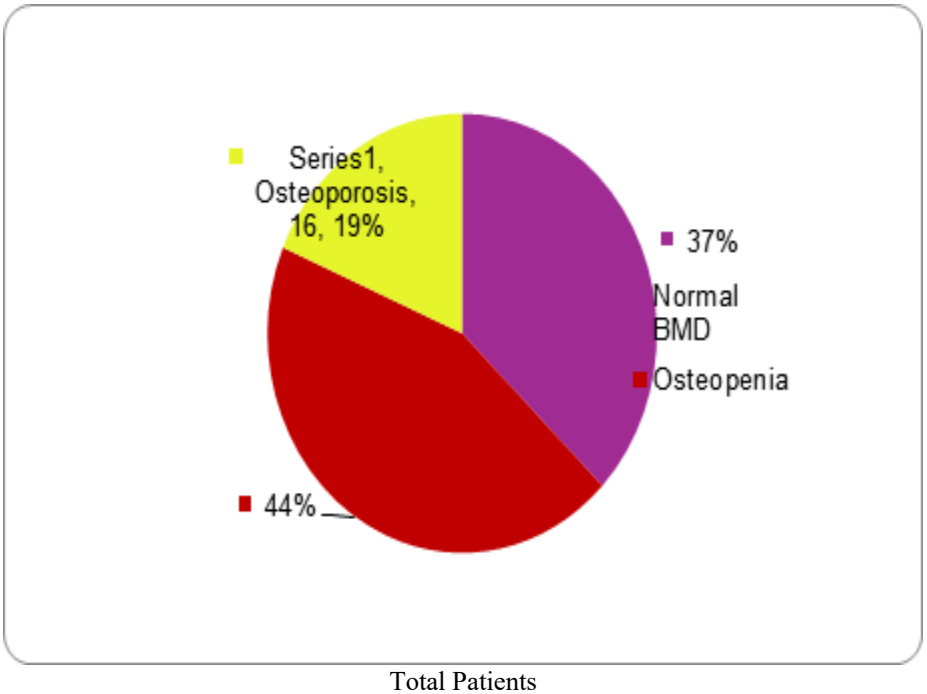
Severe osteoporosis – T-score -2.5 and below with a history of fracture.

Observations and Results:

Table 1: Comparison between different treatment groups

		Total Patients	Treatment naive	AZT based regime	TDF based regime	P value
1.	Total	86	17	25	44	0.001
2.	Mean age	37.23±7.59	35.40±	35.88±7.62	38.68±8.29	0.68
3.	Mean BMI	18.60±3.98	20.15±5.65	17.79±3.42	18.46±3.39	0.80
4.	female	37	08	10	19	0.90
5.	Male	49	09	15	25	0.90
6.	No Addiction	52	08	17	27	0.53
7.	H/o Addiction	34	09	08	17	
8.	Rural	47	10	13	24	0.90
9.	Urban	39	07	12	20	
10.	Serum ALP	159.61±145.77	203.76±236.22	131.12±80.10	158.75±128.11	0.26
11.	Serum TSH	2.94±1.75	3.12±1.45	2.95±1.90	2.86±1.79	0.44
12.	Nadir CD4	244.59±162.62	463.17±102.87	193±110.47	189.45±134.10	0.38
13.	Present CD4	389.06±195.89	485.52±113.06	350.28±194.47	373.84±212.61	0.37
14.	Serum Vit.D	35	15	10	10	0.003

		Total Patients 86	Treatment naïve 17	AZT based regime 25	TDF based regime 44	P value
15.	Normal BMD	32/86 (37.21%)	13/17 (76.47%)	06 (24%)	13/44 (29.55%)	0.21
16.	Osteopenia	38/86 (44.19%)	04/17 (23.53 %)	15 (60%)	19/44 (43.18%)	0.008
17.	Osteoporosis	16/86 (18.60 %)	0	04 (16%)	12/44 (27.27 %)	0.08
	Total	86	17	25	44	



Total Patients

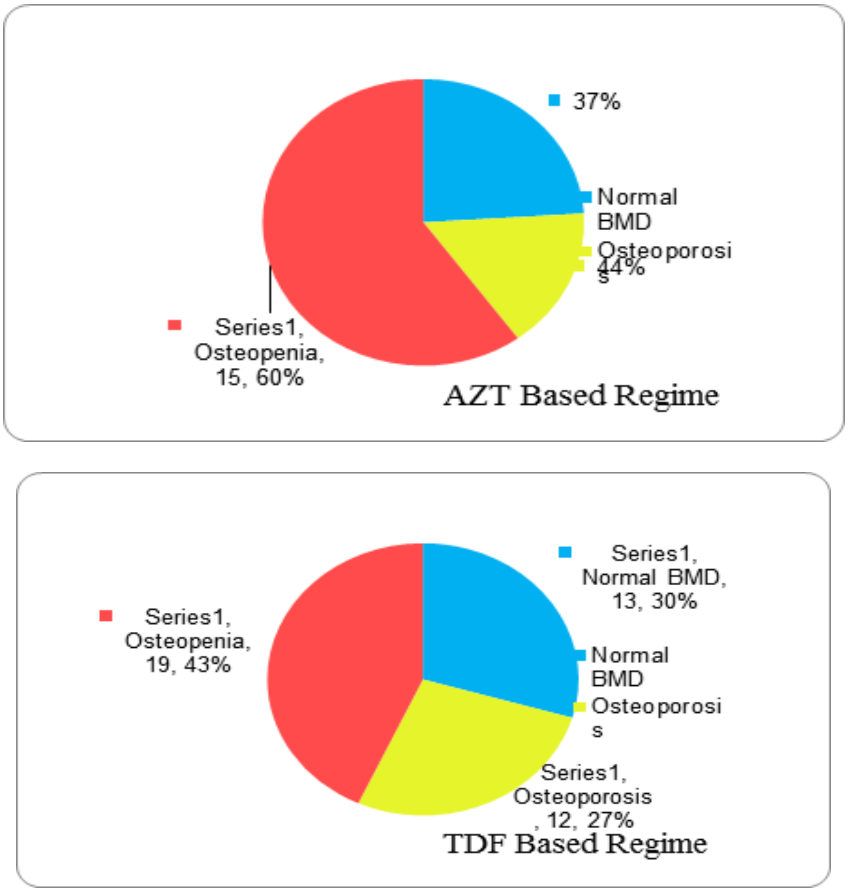


Table 2: BMD with duration of cART

Duration in Months	Total	Normal BMD	Osteopenia	Osteoporosis	P-value
24	48	19 2.73±1.19	24 9.87±5.62	5 17±3.31	
24-48	15	Nil	0731.14±7.71	08 32.12±9.12	
48	06	Nil	0364.33±14.64	0368.33±1.15	
Total	69	2.63±1.16	19.05±18.01	34.18±19.80	0.001

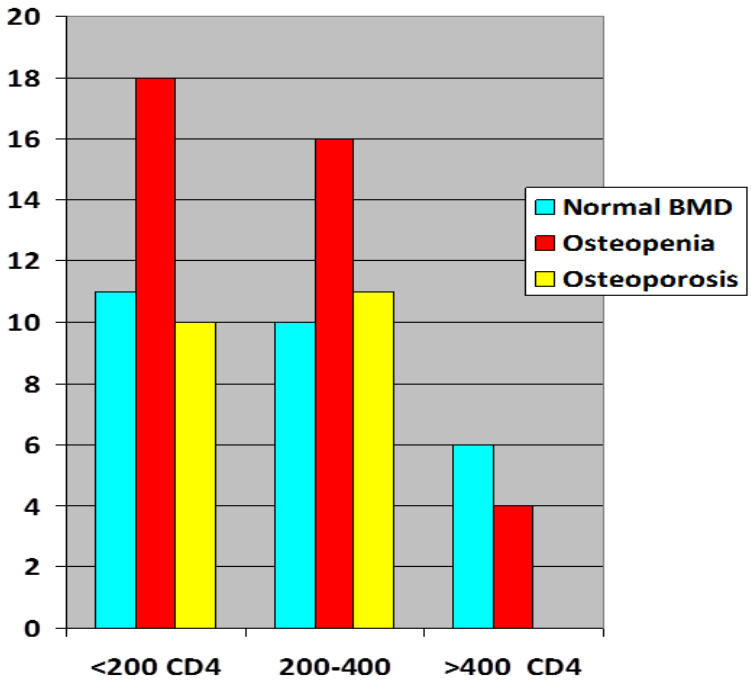


Figure 1: Distribution of BMD according to Nadir CD4 counts

Discussion

Gradual demineralization of bone is a normal feature of aging. Men and women naturally begin to lose bone around 35 year's age, at a rate of 0.5±1% per year. Women lose bone at an acceralated rate after menopause. Bone densitometry reports refer to z-or t-score. The z-score (which uses age-matched controls) compares the patients with a population adjusted for age, race and sex; the t-score (using young normal controls) compares the patient with a sex-adjusted population at peak bone mass. [10] Bone densitometry is a widely accepted tool to access bone mineralization. For an individual patient Osteopenia (t-score between -1.0 and -2.5) carries a two-fold increase in risk for fracture compared with normal BMD, and osteoporosis (t-score ≤ -2.5, without fracture) carries a four to five fold increase in fracture risk. Severe osteoporosis (t-score ≤ -2.5 plus the presence of a fracture) increases the risk for further fractures by 20 times [23-25].The study evaluated the prevalence of low BMD in HIV infected patients using either AZT based (n= 25) or TDF based (n=44) regime in the population of western Rajasthan, patients who were diagnosed as HIV infected but were treatment naïve (n=17) were also included in the study. The prevalence of Osteopenia and osteoporosis in the study population, who had a mean age of 37.23±7.59, was 44.18% and 18.6% respectively which is significantly higher compared to the normal healthy Indian population. The study indicated that HIV-infected individuals are at a greater risk of Osteopenia or osteoporosis, which may be due to interplay of multiple factors such as bone loss intrinsic to HIV infection due to immune dysfunctions [22,26] or direct stimulation of osteoclasts formation through the effect of the virus on TNF-α [27] and down regulation of RANK [28]. The study population had lower mean BMI of 18.60±3.98 which is significantly lower compared to the general healthy population, there was no statistically significant BMI differences in the different treatment groups ($p>0.3$). There was also no statistical relation between serum TSH in study subjects and their BMD values with $p>0.84$. The serum Vit D level was accessed in total of 35 patients, in which there was a statistically significant low Vit D levels in AZT and TDF based regimes ($p=0.003$) when compared to treatment naïve groups, but there was no significant difference ($p>0.2$) when the Vit D was compared to the BMD values. There was significant relation between low BMD and low nadir CD4 counts ($p<0.05$), longer duration of treatment with cART was also associated with low BMD ($p<0.001$) and there was no significant difference of treatment duration between AZT based and TDF based regime ($p=0.8$). Among the three treatment groups, TDF based regime group (n=44) was found to have higher prevalence of osteoporosis (n=12; 27.27%) compared to the AZT based (n=4; 16%) and treatment naïve groups (n=0). But Osteopenia was more prevalent in AZT based regime (n=15; 60%) compared to TDF based (n=19; 18%) and treatment naïve group (n=4; 23.53%). Treatment naïve group were also found to have high prevalence of Osteopenia (n=4; 23.53%) which may suggest that uncontrolled viremia leads to a state of systemic inflammation which impair the bone remodelling [29]. The higher prevalence of low BMD in the AZT and TDF and the higher prevalence seen with longer duration suggest that anti-retroviral drugs induce marked and significant decreased in bone mass. The exact mechanism of ART induced bone loss and the extent of their causality is not well understood and a matter of debate, however TDF may induce bone loss indirectly by proximal tubular toxicity resulting and phosphate wasting and increased bone turnover [30]. Whereas AZT may have osteoclastogenic effect on the bone attributed to increased TRAP promoter and NF-Kb [31], AZT may also induce mitochondrial DNA damage and increased oxidative stress [32] which also promotes bone loss while other refute such mechanism [33].

Summary

Osteopenia was found in 38(44.18%) patients, osteoporosis in 16(18.6%) patients and 32(37%) were having normal BMD which suggest that there is a high prevalence of low BMD in HIV infected patients. The mean nadir CD4 count among the study subjects was 244.59±162.62. Patients with low BMD (both osteopenia and osteoporosis combined) had mean nadir CD4 count low(215.98±141.51) compared to nadir CD4 count of patients with normal BMD (292.82±185.59) which was statistically significant($p<0.05$months for AZT based regime and 17.68±18.83 months for TDF based regime. Patients who were on treatment for >48 months with either of the regime had statistically significant lower BMD ($p<0.05$) as compared to those who were on treatment for 24-48 months. As the duration of treatment increases, the prevalence of low BMD also goes to increase. Among the three groups, 12(27.27%) patients who were on TDF based regime had osteoporosis as compared to 4 (16%) on AZT based regime while no osteoporosis was seen in treatment naïve group. This suggests that treatment with either of cART regimes is associated with osteoporosis. In the treatment naïve group, 13(76.47%) have normal BMD and 4(23.53%) have osteopenia suggesting that low BMD is common even in HIV subjects who are not on treatment with cART.

Therefore, HIV infection itself may be a risk factor for low bone mass. Serum vit D level in both the AZT and TDF based regime groups was lower compared to the treatment naïve group, which was

statistically significant ($p<0.05$) whereas there was no statistically significant different Vit. D level among those with normal BMD and those with low BMD ($p>0.05$). BMD decreases further after ART initiation, with older age, low BMI, low nadir CD4 count and longer duration of treatment further increasing the risk. In our study, even though bone loss was seen in treatment naïve subjects, AZT and TDF were associated with higher prevalence of Osteopenia and osteoporosis. As more number of HIV patients receive cART compared to past, the incidence of Osteopenia/Osteoporosis is expected to rise.

Conclusion and Recommendation:

We recommend that all HIV infected patients above 40 years age who are on cART for at least 1 year, should undergo DEXA scan and if result suggest osteoporosis, medical therapy should be started at the earliest with drugs available for osteoporosis. HIV patients on treatment with TDF who have osteoporosis should be evaluated for phosphate wasting and when phosphate wasting is diagnosed, discontinuation of TDF should be considered. Vit. D deficiency is prevalent in HIV-infected individuals. Higher dose of Vit. D and calcium is recommended in HIV population, particularly those on antiretroviral drugs which have demonstrated adverse effect on Vit. D metabolism and/or the bone mass.

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