Original Article

Bone health in HIV-infected children on antiretroviral therapy: An Indian study

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Abstract

Aim: The aim of this study is to determine the bone health in HIV-infected children on antiretroviral therapy (ART). **Materials and Methods:** This cross-sectional study was carried out in 31 HIV-infected children aged 5–18 years. Each patient underwent testing for serum calcium, phosphorous, alkaline phosphatase, and 25(OH) Vitamin D. Bone mineral density (BMD) was done using a DXA scanner. Patients' z scores for BMD of the lumbar spine and left femoral neck were noted. The factors associated with low BMD were analyzed. **Results:** Seven (22.6%) children had a low spinal BMD and 6 (19.4%) had low femoral neck BMD. Low serum calcium was seen in 6 (19.4%) patients and high alkaline phosphatase was seen in 15 (48.4%) patients. Low serum 25 (OH) Vitamin D levels were present in 30 (96.8%) patients, whereas all the patients had normal serum phosphorous. Duration of ART in those with low spinal BMD was 4.6 ± 3.4 years as compared to 6.4 ± 3.2 years in those with normal spinal BMD ($P = 0.235$) and for low left femoral neck BMD was 3.9 ± 2 years as compared to 6.5 ± 3.4 years for those with normal femoral neck BMD ($P = 0.031$). Mean 25(OH) Vitamin D levels were 8.4 ± 2.8 ng/ml in those with low femoral neck BMD as compared to 13.6 ± 8.3 ng/ml in those with normal femoral neck BMD ($P = 0.015$). Type of ART did not have any association with low BMD. **Conclusion:** Over 95% of HIV-infected children have low 25(OH) Vitamin D levels which affect the appendicular BMD. BMD is affected more in children who have been on ART for a shorter time. No particular ART regimen is associated with low BMD.

Key words: Antiretroviral therapy, bone mineral density, children, HIV, India, Vitamin D

INTRODUCTION

The bone health of a child depends on many interdependent factors such as nutrition, gender, socioeconomic status, presence of an illness, and history of drug intake. Bone health depends on various minerals such as calcium, phosphorus, magnesium, and even Vitamin D.[1] Low-bone mineral density (BMD) is highly prevalent among HIV-infected patients. In one meta-analysis, the prevalence of osteoporosis was three times higher among the HIV-infected patients, especially among those receiving antiretroviral therapy (ART).[2] The reduced BMD in uncontrolled HIV-infected children can be attributed to a decrease in the levels of calcitropic hormones, growth hormone, and androgens, while in children on ART, it can be attributed to increased bone turnover due to the metabolic complications of ART.[3] The cause of

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low BMD can be differentiated by the presence of clinical improvement due immune recovery and control of viral replication in children on ART.[3] Similar studies conducted in an adult population had contrasting results varying from an increase in BMD in patients on protease inhibitors to no change in BMD to a decrease in BMD.[4] Increased fracture rates in HIV-infected population are also reported with rates 30%–70% higher those in unmatched controls.[5] Vitamin D deficiency is rampant in HIV-infected children.[6] Protease inhibitors are implicated in reduction BMD in patients on ART. Nelfinavir, saquinavir, ritonavir, and indinavir have been shown to increase osteoclastic activity; lopinavir and nelfinavir decrease calcium deposition due to reduction in osteoblast alkaline phosphatase activity and osteoprotegerin expression.[7]

We undertook this study to determine the bone health in HIV-infected Indian children on ART.

MATERIALS AND METHODS

This prospective study was carried out in a Pediatric HIV clinic at a tertiary care children hospital. This study was conducted in 31 HIV-infected children aged 5–18 years who were on ART. Sample size was calculated by the universal sampling. All the HIV-infected patients who had a concomitant co-infection such as TB, Hepatitis B, or Hepatitis C were excluded from the study as these illnesses also affect the bone health. This study was conducted over a period of 2 years from November 2016 to September 2017. The study was approved by the Institutional Ethics Committee before the commencement of the study. Prior informed consent was obtained from parents/guardians. A detailed clinical evaluation of these patients was done. The mode of transmission of HIV in these patients as well as duration of the disease was determined. The World Health Organization (WHO) staging of the HIV disease (WHO disease staging) at the first presentation to the clinic was done in all the patients. The duration of ART and the current as well as past ART regimen in these children, history of intake of any additional drugs that might affect the BMD of the patient, and the initial (prior to starting ART) and the most recent CD4 count and viral load of each patient within 3 months of the BMD were noted. Each patient underwent testing for serum calcium, phosphorous, alkaline phosphatase, and 25 (OH) Vitamin D. Hypocalcemia was determined if serum calcium was <8.8 mg/dl, hypophosphatemia was determined if serum phosphorus <3.0 mg/dl, and high alkaline phosphatase was determined if the value was >180 IU/ml. A 25 (OH) Vitamin D level of <20 ng/dl was considered as deficient, between 20 and 30 ng/dl was considered as insufficient and >30 ng/dl was considered as sufficient.

Anthropometry of each patient was done by same observer. Standing height was measured to nearest 0.5 cm using stadiometer. Weight was measured on a physician balance beam scale to the accuracy of 100 gm. Height, weight, and body mass index (BMI) z scores were calculated using the Indian reference charts recommended by the Indian Academy of Pediatrics, and values above the 85th centile were considered as high BMI and those below the 5th centile were considered as low BMI.[8]

Each patient underwent a DXA scan to determine their BMD. The BMD was done using a DXA scanner, Lunar iDXA system (analysis version: 16 (SP1) manufactured by (GE Healthcare, Madison, Wisconsin, USA) by Bone Health Clinic, subsidiary of National Institute for Research in Reproductive Health Parel, Mumbai. Bone mineral content (BMC), bone area and BMD for the total body, lumbar spine, and left femoral neck were measured. Daily quality assurance scans ensured that the coefficient of variation was <0.2. All scans and scan analysis were performed by the same operator. The effective radiation dose for Lunar IDXA system has been reported as 3 μgy for total body, 37 μgy for spine, and 37 μgy for femur. Patients' z scores for BMD as well as BMC of the lumbar spine, left femoral neck, and total body along with the total body area were noted. These results were then compared to the age-matched z scores, BMD and BMC of lumbar spine, left femoral neck, total body, and total body area using the age-matched values.[9]

Statistical analysis

The factors associated with low BMD such as age, gender, type of ART, biochemistry and Vitamin D levels, and duration of ART were calculated. Proportions were analyzed by Fischer's exact test, and nominal data were calculated by *t*-test. P <0.05 was considered statistically significant.

RESULTS AND ANALYSIS

The profile of the cohort is described in Table 1. The mean age of patients was 13 ± 3 years, with an age range of 5–15 years, of which 29% were <12 years and 71% were >12 years of age. Mode of transmission of HIV was the vertical transmission in all these children. Male:Female ratio was 20:11. Mean BMI was 17.6 ± 3.3 Kg/m^2 with range of 11.4–27.7 kg/m^2. Eight (25.8%) had

low BMI, 21 (67.7%) had normal BMI, and 2 (6.5%) had high BMI. The WHO stage at the age of the first presentation to the clinic revealed 7 (22.6%) children to be in Stage 1, 1 (3.2%) child in Stage 2, 18 (58.1%) children in Stage 3, and 5 (16.1%) children in Stage 4. The mean recent CD4 count was 891.8 ± 491.3 cells/cu.mm with the range of 352–2833 cells/cu.mm. Recent viral load was available for three patients, and all had an undetectable HIV viral load. Three (9.7%) of the children suffered from fracture of the linear bones. The mean duration of ART regimen in these patients was 6 ± 3.31 years. The initial ART regimen at onset and current ART regimen is given in Table 2. The mean duration of current ART regimen was 4.9 ± 2.9 years. In the current ART regimen, 28 (90.3%) were on nonnucleoside reverse transcriptase inhibitor-based regimen and 4 (9.7%) were on protease-inhibitor (PI)-based regimen. The mean serum calcium level was 9.3 ± 0.43 mg/dl; mean phosphorus level was 4.36 ± 0.94 mg/dl; mean alkaline phosphatase level was 247.7 ± 131.1 IU/L; and mean serum 25 (OH) Vitamin D level were 12.6 ± 7.8 ng/dl. Low calcium was seen in 6 (19.35%) patients and high alkaline phosphatase was seen in 15 (48.4%) patients. Low serum 25 (OH) Vitamin D levels were present in 30 (96.8%) patients, whereas all the patients had normal serum phosphorous levels. The average lumbar spine BMD was 0.8 ± 0.2 and lumbar BMD z score was −1.2 ± 1 with the range of −2.9–+0.9. The average left femoral neck BMD was 0.84 ± 0.15 and left femoral neck BMD z score was −0.62 ± 1.03 with the range of −2.6–+1.8. Low spine BMD was seen in 7 (22.6%) patients and low left femoral BMD was seen in 6 (19.4%) patients. BMD spine had no significant association with gender, age, type of ART, duration of total ART, duration of current ART, Vitamin D levels, BMI, calcium levels, alkaline phosphatase levels, duration of Zidovudine (AZT)-based regimen, and duration of tenofovir disoproxil fumarate (TDF)-based regimen [Table 3]. BMD left femoral neck had statistical significance with duration of total ART (P = 0.031) and Vitamin D levels (P = 0.015), whereas there was no significant association with gender, age, type of ART, total ART, duration of current ART, BMI, calcium levels, alkaline phosphatase levels, duration of AZT-based regimen, and duration of TDF-based regimen [Table 4].

Table 1: Profile of cohort

Factor	Mean±SD (Range)	Factor	N(%)
Age (years)	13±3 (5-15)	Age (years)	
		<12>12	9 (29) 22 (71)
		Gender	
Duration of current ART (years)	4.9±2.9	Male Female	20 (64.5) 11 (31.4)
		BMI (kg/m^2)	
		Low	8 (25.8)
Recent CD4 count (cells/cumm)	891.8±491.3 (352-2833)	Normal	21 (67.7)
		High	2 (6.5)
		WHO stage at presentation	
BMI (kg/m^2)	17.6±3.3 (11.4-27.7)	Stage 1	7 (22.6)
		Stage 2	1 (3.2)
		Stage 3	18 (58.1)
		Stage 4	5 (16.1)
		Stage 5	18 (58.1)

ART=Antiretroviral therapy; WHO=World Health Organization; BMI=Body mass index

Table 2: Antiretroviral therapy regimen in the patients

Initial ART regimen in the patients		Current ART regimen	
ART regimen	*n* (%)	ART regimen	*n* (%)
AZT+3TC+NVP	15 (48.4)	AZT+3TC+NVP	15 (48.4)
TDF+FTC+EFV	1 (3.2)	TDF+FTC+EFV	1 (3.2)
AZT+3TC+EFV	8 (25.8)	AZT+3TC+EFV	8 (25.8)
TDF+3TC+EFV	3 (9.7)	TDF+3TC+EFV	4 (12.9)
ABC+3TC+EFV	2 (6.5)	TDF+FTC+ATZr	1 (3.2)
d4T+3TC+EFV	1 (3.2)	ABC+3TC+LPVr	1 (3.2)
d4T+3TC+NVP	1 (3.2)	AZT+3TC+LPVr	1 (3.2)
Total	31 (100)	Total	31 (100)

AZT=Zidovudine; 3TC=Lamivudine; EFV=Efavirenz; d4T=Stavudine; NVP=Nevirapine; ABC=Abacavir; TDF=Tenofovir disoproxil fumarate; ART=Antiretroviral therapy; FTC=Emtricitabine

DISCUSSION

In our study, 22.6% children had a low spinal BMD and 19.4% had low femoral neck BMD which is similar to most other studies where low BMD was seen in 4%–32% vertically HIV-infected patients.[10] However, most of these patients were not on ART. Cross-sectional studies of HIV-infected patients on ART have reported the prevalence of osteopenia ranging from 22% to 65% and of osteoporosis from 3% to 33%.[11] In children, also ART had been found to decrease the BMD.[12] However, most of these studies compared the BMD in HIV-infected children with healthy controls or controls that were HIV infected but with normal BMD. We did not take healthy controls in our study. Instead, we compared BMD with age-matched normal values as per the Indian charts.[9]

First-line ART has been associated with a 2%–6% decline in BMD during the first 12 months.[13] Longitudinal studies have shown that BMD remains relatively stable in the treatment experienced patients.[13] The decline in BMD after initiation of ART

Table 3: Factors affecting lumbar spine bone mineral density

Factor	Low BMD spine (*n*=7), *n* (%)	Normal BMD spine (*n*=24), *n* (%)	*P*
Duration of total ART (years)	4.6±3.4	6.4±3.2	0.235
Duration of current ART regimen (years)	3.4±2	5.3±3	0.075
25 OH Vitamin D level (ng/ml)	9.8±6.8	13.4±8	0.262
BMI (kg/m^2)	17.12±1.8	17.71±3.6	0.577
Calcium (mg/dl)	9.3±0.42	9.3±0.47	0.827
Alkaline phosphatase (IU/L)	279.5±120.8	238±120.8	0.564
Duration of AZT-based regimen	5±4.03	6.4±3.16	0.497
Duration of TDF-based regimen	3.75±1.76	6.7±4.5	0.309
Type of ART			
TDF + FTC/3TC + EFV	2 (40)	3 (60)	
PI based	2 (66)	1 (33)	
Type of ART			
AZT based	5 (20.8)	19 (79.16)	0.60
TDF based	2 (33)	4 (66.66)	
Gender			
Male	5 (25)	15 (75)	1
Female	2 (18)	9 (81.8)	
Vitamin D levels			
Insufficient	1 (14.2)	6 (85.7)	0.652
Deficient	6 (26)	17 (73.9)	
Low BMI	1 (12.5)	7 (87.5)	0.64
Low calcium	2 (33.3)	4 (66.6)	0.6
High alkaline phosphatase	4 (26.6)	11 (73.3)	0.68

ART=Antiretroviral therapy; BMI=Body mass index; AZT=Zidovudine; TDF=Tenofovir disoproxil fumarate; BMD=Bone mineral density; 3TC=Lamivudine; EFV=Efavirenz; NVP=Nevirapine; ABC=Abacavir; PI=Protease-inhibitor; FTC=Emtricitabine

Table 4: Factors affecting left femoral neck bone mineral density

Factor	Low BMD (*n*=6)	Normal BMD (*n*=25)	*P*
Duration of total ART (years)	3.9±2	6.5±3.4	0.031
Duration of current ART (years)	3.9±2	5.1±3.1	0.256
25 OH Vitamin D level (ng/ml)	8.4±2.8	13.6±8.3	0.015
BMI (kg/)	16.7±1.7	17.8±3.6	0.287
Calcium (mg/dl)	9.2±4.9	9.3±4.2	0.592
Alkaline phosphatase (IU/L)	303.2±172.8	234.4±119.7	0.390
Duration of AZT-based regimen	3.7±2.2	6.63±3.3	0.40
Type of ART, *n* (%)			
AZT+3TC+NVP/EFV	5 (21.7)	18 (78.2)	
TDF+FTC/3TC+EFV	1 (20)	4 (80)	
PI based	0	3 (100)	
Type of ART, *n* (%)			
AZT based	5 (20.8)	19 (79.1)	1
TDF based	1 (16.6)	5 (83.3)	
Gender, *n* (%)			
Male	4 (20)	16 (80)	1
Female	2 (18.8)	9 (81.8)	
Vitamin D levels, *n* (%)			
Insufficient	0	7 (100)	-
Deficient	6 (26)	17 (73.9)	
Low BMI, *n* (%)	1 (12.5)	7 (87.5)	1
Low calcium, *n* (%)	2 (33.3)	4 (66.7)	0.56
High alkaline phosphatase, *n* (%)	4 (26.6)	11 (73.3)	0.394

BMI=Body mass index; AZT=Zidovudine; ART=Antiretroviral therapy; BMD=Bone mineral density; 3TC=Lamivudine; EFV=Efavirenz; NVP=Nevirapine; TDF=Tenofovir disoproxil fumarate; FTC=Emtricitabine

is more pronounced in the 1st year. It was found that effective treatment of HIV infection and suppression of viral replication were crucial to the reversal of HIV-induced detrimental effects on BMD in children and may allow normal bone development.[13] This was similar to our study in which most of the children were found to have a normal BMD which could be attributed to the longer duration of exposure to ART and the ones who had lower left femoral neck BMD actually had received ART for a shortened duration which was statistically significant. However, since we did not have HIV viral load in all patients, it is not possible to comment whether HIV viremia was one of the causes of low BMD in these patients.

Patients with compromised nutritional status such as BMI in borderline percentiles have shown to have lower BMD z scores and BMC values.[10] In our study, 87.5% patients with normal BMI had a normal spine BMD and normal left femoral neck BMD. Similarly, in a study by Hansen *et al*., most of the patients had normal weight and no association was found between BMI and low BMD.[14]

In our study, three patients were on PI-based regimen of which two had low spine BMD. Whether this is due to PI or due to the concomitant NRTI remains inconclusive and more patients on PI-based

regime need to undergo BMD analysis to determine whether this is direct causal association or a chance association. Studies on PI-based regimen are found to have a greater bone loss in adults.[15]

The use of TDF is known to cause low bone mass.[16] TDF is a phosphonate, it is conceivable that it could be selectively taken up by osteoclasts by a mechanism similar to that of bisphosphonates, ultimately causing cellular stress. The resulting cellular stress would likely perturb cellular DNA synthesis (i.e., nuclear and/or mitochondrial) and gene expression.[16] The loss of bone density due to TDF exposure could also be associated with tenofovir-induced renal dysfunction, particularly renal proximal tubule dysfunction.[16] In our study, only six patients were on TDF-based regimen while majority were on AZT based regimen. Furthermore, BMD loss was not significantly greater in the ones who were on TDF. Hence, it is not possible to comment whether a particular type of antiretroviral drug is associated with a lower BMD on the basis of our study.

Among other anti-retroviral medications, efavirenz has been consistently associated with Vitamin D deficiency. Efavirenz affects Vitamin D metabolism by inducing enzyme 24-hydroxylase which inactivates Vitamin D by converting it to its inactive form (calcitroic acid), thereby causing bone loss. In our study, low serum 25 (OH) Vitamin D levels were seen in 96.8% of the patients. In many other studies, Vitamin D deficiency has been noted in HIV-infected patients ranging from 23% to 92%[17] Poor nutrition and chronicity of the illness can be a reason for low Vitamin D levels in these patients. HIV-infected patients may have impaired 1α-hydroxylation that decreases the production and action of the active Vitamin D metabolite 1,25 (OH2D3) despite normal Vitamin D levels.[17] In our study, the Vitamin D levels were lower in patients with low left femoral neck BMD suggesting that Vitamin D deficiency affects the appendicular skeleton more than the axial skeleton. This could be due to the fact that the hip has more cortical bone than the lumbar spine, which has mainly trabecular bone, and as bone remodelling is more rapid for trabecular than for cortical bone,[18] a new balance between formation and resorption may take longer to occur in the hip.

Although low calcium was seen 19.35% and high alkaline phosphatase was seen in 48.4% of these patients, we did not find any association between these biochemical parameters and low BMD. Similar findings have been noted in other studies.[19] The low calcium and high alkaline phosphatase could also be related to low Vitamin D levels in these patients.

Among our study participants, 10% patients suffered from fractures. However, all of them had normal BMD. Thus, although low BMD is associated with a higher fracture risk, patients with HIV can still develop fractures even if the BMD is normal.[17] We did not find any association of age and gender with the BMD.

Although bisphosphonates are known to increase the BMD in patients with osteoporosis, whether they are required in HIV-infected children with low BMD remains undetermined. A systematic Cochrane review performed in 2007 identified three randomized controlled trials of alendronate in patients with HIV infection and low BMD values, the review indicated a favorable effect on BMD values after 1 year of alendronate therapy continued with Vitamin D and calcium supplementation. However, this review was mainly in adult patients.[20] However, since it is known that BMD normalizes with the longer duration of ART and the fractures in children with HIV are not related with BMD, the role of alendronate in HIV-infected children with low BMD still remains controversial.

Limitation of our study

It was a cross-sectional study, and hence, we were unable to evaluate the change of bone mass over a period of treatment. Prospective, longitudinal study is therefore warranted. We did not do BMD in ART naïve patients and hence could not determine the effect of HIV on bone health *per se*. Many children had experienced various ART regimens and therefore our ability to evaluate the impact of specific antiretroviral medications was limited. Our study was not a case-controlled study. The BMD values were compared with the Indian charts.

CONCLUSION

BMD is low in 22% of Indian children on ART. Most of these patients also have a low Vitamin D level. BMD is affected more in children who have been on ART for a shorter time as compared to those who have been on ART for a longer time. Vitamin D supplementation may play a role in preventing low BMD in children on ART in the short term. Whether Vitamin D supplementation helps to improve bone health in these children needs to be analyzed by intervention studies.

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Conflicts of interest

There are no conflicts of interest.

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