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RESEARCH ARTICLE

Prevalence of Bone Loss and the Short-Term Effect of Anti-retroviral Therapy on Bone Mineral Density in Treatment-Naïve Male Japanese Patients with HIV

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Abstract:

Background:

There have been few studies have shown the relationship between HIV and low Bone Mineral Density (BMD) in Asian countries. In particular, research on the early impact of anti-HIV drugs on BMD is scarce.

Objective:

We studied the prevalence of bone loss and changes of BMD after the start of Anti-Retroviral Therapy (ART) in Japanese naïve patients with HIV.

Methods:

Male patients with HIV who visited our hospital between 2010 and 2016 were enrolled. Patients underwent BMD analyses before and one year after ART. Changes in BMD after ART initiation were evaluated by paired t-tests. To identify clinical factors affecting BMD after ART initiation based on the BMD change ratio, multiple regression analysis was performed.

Results:

Thirty-one patients were followed up. By employing the T-scores in the lumbar spines and femoral necks, the prevalence of osteopenia and osteoporosis was found to be 38.7-45.2% and 6.2% respectively. There were significant BMD decreases after ART initiation. Use of Tenofovir Disoproxil Fumarate (TDF) / emtricitabine (FTC), use of Protease Inhibitors (PIs), and low CD4 cell counts were independent risk factors for lumbar spine BMD decrease. Urinary N-terminal telopeptide / creatinine was the independent risk factor for femoral neck BMD decrease.

Conclusions:

Low BMD was prevalent in our study cases. Low CD4 cell counts at the onset of ART initiation, TDF/FTC use, and PI use increased the risk of lumbar spine BMD decrease significantly more, while ART affected femoral neck BMD of patients with higher bone metabolic activity significantly more.

Keywords: HIV, Bone Mineral Density (BMD), Tenofovir (TDF)/emtricitabine (FTC), Protease Inhibitor (PI), Osteopenia, Osteoporosis, Urinary N-terminal Telopeptide (NTx), Creatinine.

Article History

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1. INTRODUCTION

Over the past two decades, HIV treatment has developed, and the mortality of HIV-infected individuals has improved dramatically. As life expectancy has increased, several comorbidities have emerged. Liver disease, renal dysfunction, cardiovascular disease, diabetes mellitus occur more frequently

in HIV patients. It is now also well known that low bone mass is prevalent in HIV patients [1].

The causes of low bone mass are considered to be multifactorial and include HIV infection itself, age, Body Mass Index (BMI), renal dysfunction, female sex, vitamin D deficiency, hepatitis coinfection, and others. Anti-HIV drug use is also important [2]. Some studies showed that race was one of the risk factors for bone fracture in HIV patients [3, 4].

Many studies in North America, Europe, and Oceania have shown the relationship between HIV and low bone mass, but

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there have been few such studies in Asian countries, and these were mainly conducted as retrospective cross-sectional studies. In particular, research on the early impact on Bone Mineral Density (BMD) of recent anti-HIV drugs is scarce in Asian countries.

In this study, the focus was on male Japanese treatment-naïve HIV patients, in whom the prevalence of bone loss and the short-term effect of Anti-retroviral Therapy (ART) on bone mineral density was evaluated, and the clinical factors related to BMD loss were examined using a historical cohort analysis.

2. METHODS

This study was approved by the ethics committee of Teikyo University School of Medicine (13-1390). HIV patients who visited the outpatient clinic of Teikyo University Hospital, Tokyo, Japan between April 2010 and October 2016 were enrolled to this study. Written, informed consent was obtained from all study participants. Patients underwent BMD analyses twice, first, before the start of ART, and second, 44-56 weeks after the start of ART with the same dual energy X-ray absorptiometry scanner (Discovery SI, Hologic Inc, Bedford, MA). Both lumbar spine BMD and bilateral femoral neck BMD were analyzed. The BMD value of the lumbar spine and the average BMD value of the femoral necks were used to calculate T-scores. T-scores were calculated as comparisons with young normal reference values expressed as standard deviation units. Referred to the criteria of the World Health Organization (normal: T-score of -1.0 or higher, osteopenia: between -1.0 and -2.5, osteoporosis: -2.5 or lower), patients were categorized into 3 groups: normal, osteopenia, and osteoporosis. Osteopenia and osteoporosis were categorized as the low BMD group, and normal was categorized as the normal BMD group.

Clinical factors, including age, body mass index, smoking status, use of corticosteroid, hepatitis C co-infections, and laboratory data (CD 4 cell counts, HIV RNA replication, aspartate transaminase (AST), alanine aminotransferase (ALT), alkaline phosphatase, serum creatinine, serum calcium, serum phosphorus, serum 1.25-dihydroxyvitamin D3 (1.25VitD), 25-hydroxyvitamin D, serum fibroblast growth factor (FGF)23, serum intact parathyroid hormone, serum bone-specific alkaline phosphatase, urinary N-terminal telopeptide/creatinine (NTx/Cr)), were evaluated. These laboratory data were measured at the same time as the first BMD analyses.

The results are expressed as medians [interquartile range], unless otherwise indicated.

Changes in BMD after the start of ART were evaluated by paired *t*-tests. The BMD change ratio was calculated by the following analysis: [(BMD after the start of ART – BMD before the start of ART)/BMD before the start of ART]* 100(%). To identify the risk factors for low bone mass in treatment-naïve patients and the factors affecting BMD after the start of ART (44-56 weeks after the initiation of ART), stepwise multiple regression analysis was performed. In addition, we compare the low BMD group with the normal BMD group to analyze the risk of BMD loss. For comparisons of independent groups, chi-square test, Fisher's exact test, or

Mann-Whitney U test was used to analyze continuous and categorical data, as appropriate. Two-sided P values were calculated for all comparisons and were considered significant at $P < 0.05$. StatFlex version 6 (Artech Co., Osaka, Japan) software was used for analysis.

3. RESULTS

Thirty-one patients were followed up (Table 1). Age was 37.0 [31.3-49.0] years old. CD4 cell count was 275 [125-347]/ μ L. HIV RNA copies was 41.0 [21.0-101] $\times 10^3$. Referred to lumbar spines T-scores, 16 (51.7%) cases were osteopenia. Referred to femoral necks T-scores, 10 (32.3%) cases were osteopenia and 1 (3.2%) cases were osteoporosis. The baseline BMD status was shown in Table 2.

Table 1. Clinical data of the study population before the start of anti-retroviral therapy.

	Normal Value Range	Median	Interquartile Range
Lumbar BMD (g/cm ²)	–	0.919	[0.856-1.054]
Lumbar T-score	–	-1.10	[-1.60-0.10]
Lumbar Z-score	–	-0.500	[-0.900-0.200]
Femoral neck BMD (g/cm ²)	–	0.759	[0.711-0.843]
Femoral T-score	–	-0.800	[-1.20--0.188]
Femoral Z-score	–	-0.350	[-0.800-0.300]
Age (years)	–	37.0	[31.3-49.0]
BMI	–	22.4	[19.6-24.1]
CD4 (/ μ L)	–	275	[125-347]
HIV RNA copies ($\times 10^3$)	–	41.0	[21.0-101]
AST (IU/L)	[13-30]	24.0	[20.0-29.5]
ALT (IU/L)	[10-42]	21.0	[15.0-29.8]
ALP (IU/L)	[124-222]	262	[202-282]
Cre (mg/dL)	[0.65-1.07]	0.800	[0.725-0.890]
Ca (mg/dL)	[8.8-10.1]	8.90	[8.63-9.20]
P (mg/dL)	[2.5-4.5]	3.60	[3.30-3.80]
1.25VitD (pg/ml)	[20-60]	46.7	[37.7-55.0]
25OHvitD (pg/ml)	[7-41]	18.5	[14.5-26.0]
FGF23(pg/ml)	[10-50]	43.0	[37.1-55.0]
PTHin(pg/ml)	[10.3-65.9]	39.0	[31.0-49.0]
BAP (μ g/L)	[3.7-20.9]	11.8	[9.40-15.0]
NTx/Cr (nmolBCE/mmolCr)	[13.0-66.2]	32.5	[28.7-43.8]
Smoking (cases)	–	13	(41.9%)
HCV infection (cases)	–	1	(3.23%)

BMD: Bone mineral density, BMI: body mass index, AST: Aspartate transaminase, ALT: Alanine aminotransferase, Ca: Serum calcium, P: Serum phosphorus, 1.25VitD: serum 1.25-dihydroxyvitamin D3, 25OHvitD: serum 25-hydroxyvitamin D, FGF23: Serum fibroblast growth factor 23, PTHin: Serum intact parathyroid hormone, BAP: Serum bone-specific alkaline phosphatase, NTx/Cr: Urinary N-terminal telopeptide/creatinine.

On stepwise multiple regression analysis with lumbar spines / femoral necks T-scores as objective variables and clinical data as potential explanatory variables, high serum AST, high serum ALT, and old age were independent risk factors for low bone mass (Table 3). Two-group comparison analysis showed that old age was the independent risk factors for low bone mass (Table 4).

Table 2. The baseline BMD status of the 31 patients.

–	–	Femoral Necks	
		Normal BMD	Low BMD
Lumbar spines	normal BMD	14(45.2%)	1(3.2%)
	low BMD	6(19.4%)	10(32.3%)

The stepwise multiple regression analysis was performed using the data of the BMD change ratio as an objective variable, and clinical data at the start of HIV treatment / the ART regimen as potential explanatory variables. This analysis showed that TDF/FTC use ($t=2.87569$, $p=0.00777$), PI use ($t=2.09614$, $p=0.04558$), and low CD4 cell counts ($t=3.77804$, $p=0.00079$) were independent factors related to decreased lumbar spine BMD, while NTx/Cr ($t=3.02340$, $p=0.00542$) was an independent risk factor for femoral neck BMD loss. Bone mineral density status before the start of ART did not affect the BMD decrease after the start of ART.

Table 3. Stepwise multiple regression analysis of risk factors for low bone mass in treatment-naïve patients.

–	–	t	P (≤ 0.05)
Lumbar BMD	Smoking	1.50690	0.14437
–	Age	-2.09502	0.00680
–	P	2.04948	0.05105
–	AST	3.48126	0.00185
–	ALT	2.63861	0.01412
Femoral BMD	Age	-4.13423	0.00031
–	AST	3.749325	0.00086
–	ALT	2.85756	0.00812

P: Serum phosphorus, AST: Aspartate transaminase, ALT: Alanine amino-transferase.

Table 4. Two-group comparison to analyze the risk factors of low bone mineral density based on the lumbar spines.

–	Normal BMD (n=15)		Low BMD (n=16)		P (Univariate)	P (Multivariate)
Age (years)	33.0	[27.3-37.0]	48.0	[35.0-61.5]	0.0036	0.0180
BMI	22.4	[19.4-23.2]	22.4	[20.3-24.3]	0.7478	–
CD4 (μL)	198	[81-338]	287	[243-407]	0.1490	–
HIV RNA copies ($\times 10^3$)	69.0	[11.8-258]	37.5	[28.0-55.0]	0.4525	–
AST (IU/L)	20.5	[18.5-23.5]	28.0	[24.3-37.5]	0.0075	0.2658
ALT (IU/L)	20.0	[15.0-25.5]	23.0	[15.0-47.3]	0.4051	–
ALP (IU/L)	212	[174-277]	271	[254-297]	0.0214	0.2717
Cre (mg/dL)	0.800	[0.725-0.890]	0.810	[0.690-0.880]	0.4721	–
Ca (mg/dL)	8.70	[8.60-8.98]	9.00	[8.70-9.35]	0.1175	–
P (mg/dL)	3.80	[3.50-4.00]	3.35	[3.15-3.60]	0.0717	–
1.25VitD (pg/ml)	38.3	[33.1-57.9]	55.0	[43.3-68.7]	0.1018	–
25OHVitD (pg/ml)	18.0	[13.3-26.3]	19.0	[14.5-25.3]	0.8457	–
FGF23(pg/ml)	46.0	[39.0-51.0]	41.0	[35.0-59.0]	0.9226	–
PTHin(pg/ml)	34.0	[23.8-44.0]	42.0	[31.3-49.8]	0.2457	–
BAP ($\mu\text{g/L}$)	11.4	[9.7-13.5]	12.1	[8.8-15.5]	0.7890	–
NTx/Cr (nmolBCE/mmolCr)	30.0	[14.1-42.1]	34.9	[31.5-46.6]	0.1988	–
Smoking (cases)	5	(33.3%)	8	(50.0%)	0.3473	–

BMI: Body mass index, AST: Aspartate transaminase, ALT: Alanine aminotransferase, Ca: Serum calcium, P: Serum phosphorus, 1.25VitD: Serum 1,25-dihydroxyvitamin D₃, 25OHvitD: Serum 25-hydroxyvitamin D, FGF23: Serum fibroblast growth factor 23, PTHin: Serum intact parathyroid hormone, BAP: serum bone-specific alkaline phosphatase, NTx/Cr: urinary N-terminal telopeptide/creatinine.

The changes in lumbar spine and femoral neck BMDs after the start of ART are shown in Fig. (1). According to paired *t*-test, there were significant changes after initiation of ART in both lumbar spine BMD and femoral neck BMD (lumbar spine: 0.919 [0.856 - 1.055] to 0.902 [0.840 - 1.001] g/cm^2 $P=0.0004$, femoral neck: 0.760 [0.711-0.843] to 0.747 [0.714-0.809] g/cm^2 $P=0.0001$). The average BMD change ratio was -3.16% for lumbar spine BMD and -2.91% for femoral neck BMD. The BMD status of the 31 patients was not

significantly changed one year after the start of ART.

There are slight but significant changes after the initiation of ART in both lumbar spine BMD and femoral neck BMD (lumbar spine: 0.919 [0.856 - 1.055] to 0.902 [0.840 - 1.001] g/cm^2 $P=0.0004$, femoral neck: 0.760 [0.711-0.843] to 0.747 [0.714-0.809] g/cm^2 $P=0.0001$).

All cases were administered with two nucleoside reverse-transcriptase inhibitors as backbone drugs and one protease inhibitor or integrase strand transfer inhibitor as a key drug of

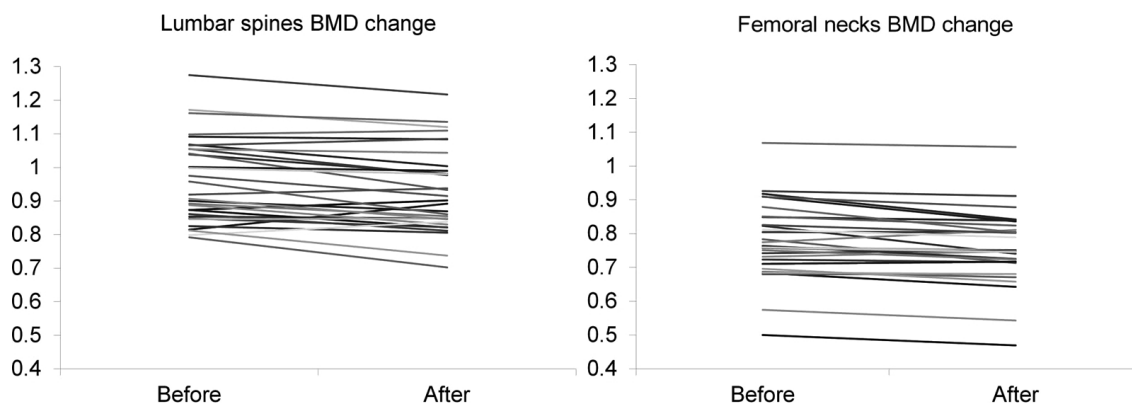


Fig. (1). Changes of lumbar spine/femoral neck bone mineral density (BMD) after the start of ART.

the ART regimen. Eighteen (58.1%) patients were treated with tenofovir disoproxil fumarate (TDF)/emtricitabine(FTC), and 13 (41.9%) patients were treated with abacavir (ABC)/lamivudine (3TC) as backbone drugs of the ART regimen. Eighteen (58.1%) patients were treated with protease inhibitors (PIs) (2 patients: atazanavir/ritonavir, 16 patients: darunavir/ritonavir), and 13 (41.9%) patients were treated with Integrase Strand Transfer Inhibitors (INSTIs) (2 patients: raltegravir, 7 patients: elvitegravir/cobicistat, and 4 patients: dolutegravir) as key drugs of the ART regimen. Seven patients were treated with ABC/3TC and PI, and 6 patients were treated with ABC/3TC and INSTI. Eleven patients were treated with TDF/FTC and PI, and 8 patients were treated with TDF/FTC and INSTI.

4. DISCUSSION

This study showed the relationship between BMD change and ART in Japanese male treatment-naïve HIV patients. These study results are of greater value in Asian clinical settings because research on the early impact on BMD of the recent anti-retroviral agents after the start of ART has been rare in Asian countries. Although there have been few previous studies of the prevalence of bone marrow loss in Asian HIV patients, no previous study involving treatment-naïve HIV patients has examined the short-term effect (about 1 year) of anti-retroviral agents, unlike the present study [5 - 9].

Even in the treatment-naïve setting, low BMD was prevalent in Japanese HIV patients. Age was the independent risk factor for low bone mass, as previously demonstrated. In the present study, elevations of serum AST and/or ALT levels were also thought to be risk factors. Liver damage induces lower production of bile, which could lead to inhibition of lipid-soluble vitamin absorption. These result in decreased serum activated vitamin D levels and inhibition of Ca absorption in the small intestine. The present result might be reflective of these mechanisms, though HBV or HCV infection and 1.25 OH-VitD were not significantly related to BMD in the present study. However, AST/ALT levels in the present population were mostly in the normal ranges. Serum AST was 24.0 [20.0-29.5] IU/L, and serum ALT was 21.0 [15.0-29.8] IU/L. These related factors might be less important than age.

About one year after the start of ART, both lumbar spine BMD and femoral neck BMD decreased significantly, which showed that anti-HIV drugs could affect bone metabolism, as

previously shown in North America and Europe.

The present results suggested that TDF/FTC use, PI use, and low CD4 cell counts before ART initiation were independent risk factors for lumbar spine BMD loss. The lumbar spines are predominantly cancellous bones, which have high bone metabolic activity. Although the mechanism of decreased BMD in patients receiving these drugs remains to be determined, anti-HIV drugs might affect the balance between bone formation and bone resorption. A study by another group in Japan, which was conducted in Japanese patients receiving ART, showed that long-term PI use was associated with bone mineral density loss [10]. Considering all of these results, including the present one, PIs use is an especially definite risk factor for BMD loss in Japanese HIV patients. A previous study also showed that CD4 cell counts were related to BMD in some patient groups [11]. Progression of HIV infection is usually reflected in a decrease of CD4 cell counts. Although it is difficult to know the precise duration of HIV infection, disease duration might be one of the risk factors for lumbar BMD loss.

NTx/Cr was the only risk factor for femoral neck BMD loss. The femoral neck is comprised mainly of cortical bone, with relatively low bone metabolism. NTx/Cr and serum osteocalcin are representative of the activity level of bone metabolism. We think that anti-HIV drugs could affect BMD in patients with high NTx/Cr levels, as seen with relatively high bone metabolism, though osteocalcin was not measured in the present study. A previous study showed that patients with advanced HIV disease demonstrated high levels of NTx/Cr [12]. These results, including the present data, also showed that progression of HIV infection might be a risk factor for femoral BMD loss, and that cART should be started as soon as possible with a view to decreasing BMD loss at both the lumbar spines and the femoral necks.

This study was conducted in only one institute with a relatively small number of subjects. More large-scale clinical trials are needed to validate this study's results. However, the present results are of particular clinical value in Asian countries. Given the present results, the choice of anti-retroviral regimens needs to be made carefully, and adequate ART should be started as early as possible.

LIST OF ABBREVIATIONS

TDF	= Tenofovir Disoproxil Fumarate
FTC	= Emtricitabine (FTC)
Art	= Nrti-retroviral Therapy
PIs	= Protease Inhibitors
BMD	= Bone Mineral Density
BMI	= Body Mass Index, AST: Aspartate transaminase
ALT	= Alanine Aminotransferase, Ca: Serum calcium
P	= Serum Phosphorus
1.25VitD	= Serum 1.25-dihydroxyvitamin D3
25OHvitD	= Serum 25-hydroxyvitamin D
FGF23	= Serum Fibroblast Growth Factor 23
PTHin	= Serum Intact Parathyroid Hormone
BAP	= Serum Bone-specific Alkaline Phosphatase, fTST
Serum free Testosterone, NTx/Cr	= Urinary N- Terminal Telopeptide/creatinine

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was approved by the ethics committee of Teikyo University School of Medicine (13-1390). HIV patients who visited the outpatient clinic of Teikyo University Hospital, Tokyo, Japan between April 2010 and October 2016 were enrolled to this study. Written, informed consent was obtained from all study participants.

HUMAN AND ANIMAL RIGHTS

No Animals were used in this research. All human research procedures followed were in accordance with the ethical standards of the committee responsible for human experimentation (institutional and national), and with the Helsinki Declaration of 1975, as revised in 2013.

CONSENT FOR PUBLICATION

Written, informed consent was obtained from all study participants.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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YY contributed to the conception; design; data acquisition, analysis and interpretation; drafting of the manuscript; and critical revision for important intellectual content.

IK, KM, KS, TK, and YO contributed to the study design and coordination, and helped draft the manuscript. All authors have read and approved the final manuscript.

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