Reduced Bone Mineral Density and Serum C-Telopeptide Concentration in HIV-infected Patients in Cipto Mangunkusumo Hospital

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ABSTRACT

Aim: *to evaluate BMD and bone resorption marker in HIV-infected patients in Cipto Mangunkusumo Hospital, Jakarta.*

Methods: *a cross-sectional study was performed between February and May 2008 in adult HIV-infected patients who had not been treated with antiretrovirals. BMD was measured at the lumbar spine by dual energy X-ray absorptiometry (DEXA) bone densitometer (Lunar Prodigy, GE Medical System, USA), whereas CTX (C-terminal telopeptide) was measured by an automated analyzer (Elecsys 2010, Roche Diagnostics GmbH, Mannheim, Germany) using the b-Crosslaps serum reagents.*

Results: *fourty-two patients were included, comprising 31 (73.8%) men and 11 (26.2%) women. Patients' median age was 28 years, ranging from 22 to 39 years old. The peak age group was 26-30 years old. Low BMD (osteopenia) was found in 11 (26.2%) of patients. Mean serum CTX level was significantly correlated with BMD (r=0.446; p=0.003).*

Conclusion: *patients with low CD4 count and low BMI tended to have higher serum CTX. HIV-infected, treatment-naïve patients possess a significant risk for reduced BMD due to increase bone resorption activity. Further studies are needed to evaluate the association of disease severity and bone resorption markers.*

Key words: *bone mineral density, serum C-telopeptide, HIV patients, CD4 count.*

INTRODUCTION

Increased risk of reduce bone mineral density (BMD) and altered bone mineral metabolism have emerged as a metabolic complications of HIV infections.[1-3] The prevalence of osteoporosis in HIV-infected persons is about 15%.[4] In normal condition, bone is constantly remodeled with bone resorption tightly coupled to bone formation. In HIV-infected patients, bone resorption is markedly increased stably or decreased bone formation.[5,6] High bone turnover favoring bone resorption may lead to reduced BMD and increase the risk of fracture.[7]

Altered bone mineral metabolism was reflected by the decreased bone formation markers and increased bone resorption markers.[8,9] Previously, biochemical markers of bone turnover have been used to evaluate HIV-infected patients receiving antiretroviral therapy (ART) since it was associated to an adverse effect on BMD.[10,11] However, the HIV infection itself may induce pro-inflammatory cytokines, such as *tumor necrosis factor alpha* (TNF-α), interleukin (IL-1) and IL-6 which increases osteoclastogenesis by increasing the production of receptor activator of nuclear factor êB ligand (RANKL).[12,13,14]

Bone remodeling disorder could be detected with bone turnover markers such as alkaline phosphatase, osteocalcin, and type I collagen degradation products. Among these markers, urinary degradation products of type I collagen (C-terminal telopeptide [CTX] and N-terminal telopeptide [NTX]) have been demonstrated to be more superior and is widely used. Recently, serum automated assay for serum CTX has been developed to detect the increase of bone resorption and predicted fracture risk.[15,16]

Our preliminary study in Cipto Mangunkusumo Hospital showed that 31.6% of adult HIV-infected patients had abnormal low BMD (osteopenia or osteoporosis).[17] However, biochemical markers of bone turnover have not been included in the assessment. This study was aimed to assess the effect of HIV infection on BMD and serum CTX level in treatment-naïve HIV-patients in Cipto Mangunkusumo Hospital.

METHODS

Study Design and Subjects

This was a cross-sectional study in the Department of Internal Medicine, Faculty of Medicine, University of Indonesia – Cipto Mangunkusumo Hospital between February and May 2008. Study population was adult, treatment naïve, HIV-infected patients in Cipto Mangunkusumo Hospital. Patients were recruited consecutively.

CTX Measurement

Fasting sera from all patients were kept in the refrigerator until ready for assay. Serum CTX was measured by an automated analyzer (Elecsys 2010, Roche Diagnostics GmbH, Mannheim, Germany) using the β-Crosslaps serum reagents. This assay is specific for cross-linked â-isomerized type I collagen C-terminal telopeptide fragments and uses two monoclonal antibodies, each recognizing the Glu-Lys-Ala-His-βAsp-Gly-Gly-Arg peptide (Crosslaps antigen). A 50 µL serum was incubated with biotinylated monoclonal antibody against the Crosslaps antigen. A second antibody labeled with a ruthenium complex is then added together with streptavidin-coated microparticles. These microparticles are then magnetically captured onto the surface on an electrode. Induction of chemiluminescent emission was generated by applying voltage on the electrode which then was measured by a photomultiplier and compared to a calibration curve. Normal values for men and pre-menopausal women were 0.025-0.573 ng/mL, whereas normal values for postmenopausal women were 0.104-1.008 ng/mL.

Bone Mineral Density (BMD) Measurement

BMD was measured at the lumbar spine by dual energy X-ray absorptiometry (DEXA) bone densitometer (Lunar Prodigy, GE Medical System, USA). The spine was measured in the postero-anterior (PA) projection and results were reported for the total spine L1-4. Osteoporosis is classified by DXA at the lumbar spine as T-score lower than -2.5, whereas osteopenia is considered to be a T-score between -1 and -2.5.

Statistical Analysis

Characteristics of the study subjects are presented descriptively. Mean differences of CTX in normal and abnormal BMD were tested using the student-t test. A p value less than 0.05 was considered statistically significant. Statistical analyses were performed by using computer software SPSS for Windows version 11.5 (SPSS Inc., Chicago, Illinois, USA).

RESULTS

There were 42 HIV-patients recruited for this study. Men were three times higher than women. Patients' median age was 28 years, ranging from 22 to 39 years old. The peak age group was 26-30 years old. The majority of patients had a history of injecting drug users as their risk of getting HIV infection. There were 31 (73.8%) patients who had low CD4 count and half of them had low body mass index. About a quarter of patients had low BMD (Table 1).

Table 1. Characteristics of The Study Subjects (n=42)

Characteristic	N	%
Sex		
▪ Men	31	73.8
▪ Women	11	26.2
Age group (years)		
▪ 21 – 25	5	11.9
▪ 26 – 30	25	59.5
▪ 31 – 35	8	19.0
▪ 36 – 40	4	9.5
Risk of HIV-transmission		
▪ IVDU	32	76.2
▪ Sexual	10	23.8
CD4 count (cells/mm^3)		
▪ > 200	11	26.2
▪ < 200	31	73.8
Body mass index (kg/m^2)		
▪ <19	21	50.0
▪ 19 – 25	18	42.9
▪ > 25	3	7.1
Bone mass density at L1-L4		
▪ Osteopenia	11	26.2
▪ Normal	31	73.8

The mean serum CTX levels were 0.52 ± 0.341 ng/mL for men and 0.56 ± 0.417 for women. This difference was not significant. A significant correlation was found between BMD and CTX level (r=0.446; p=0.003). Significant increase of mean CTX level was also observed in osteopenic compared to normal BMD of HIV-patients. Patients with low CD4 count tend to have higher serum CTX levels. There was also a tendency that patients with low BMI had higher serum CTX levels (Table 2).

Table 2. Mean Difference of CTX Levels Between Osteopenic and Normal Bone Mass Density in HIV-infected Persons

	Mean CTX level	p value (student-t test)
Bone mass density		
▪ Osteopenia (n=11)	0.73 ± 0.473	**0.033**
▪ Normal (n=31)	0.46 ± 0.283	
Body mass index (kg/m^2)		
▪ Underweight	0.64 ± 0.407	0.081
▪ Normal/ slightly overweight	0.45 ± 0.297	
CD4 count		
▪ < 200 cells/mm3 (n=31)	0.58 ± 0.395	
▪ > 200 cells/mm3 (n=11)	0.39 ± 0.156	0.117

DISCUSSION

Studies on bone mass metabolism in HIV-infected persons in Indonesia are limited. Our study results supported the effect of HIV infection on bone density metabolism among treatment naïve, HIV-infected patients. About a quarter of patients showed reduced BMD (osteopenia) on admission. A case control study in elderly subjects showed that reduced BMD was significantly higher in HIV positive individuals (59% at total hip and 67% at lumbar spine) compared to HIV negative controls (26% at total hip and 39% at lumbar spine).[18]

A recent meta-analysis that found 67% of HIV-patients had osteopenia and osteoporosis; however, this study also included patients receiving ART.[19] Studies evaluating BMD have shown conflicting results about the effect of highly active ART (HAART) that not only could decrease T-scores[20], but also could increase BMD[21] or had no effect (stable).[22] These differences of outcome may be caused by the difference of antiretrovirals used for the patients or the timing of BMD measurements.

Our data showed that the mean CTX level was near the upper limit of normal for men and premenopausal women. The level of serum CTX was significantly correlated with the BMD values. This result supports the use of serum CTX as a reliable marker for altered bone resorption among HIV-infected patients and might be offered for early clinical assessment in patients with HIV.

This current study failed to show any association between serum CTX and disease severity as reflected by CD4 count and BMI. However, there was a tendency that patients with low CD4 count and BMI had higher serum CTX level. Our study was limited by the small sample size which could not reach a sufficient power to detect those differences. Larger samples are required to confirm these hypotheses.

CONCLUSION

About a quarter of patients in this study had low bone mineral density (BMD) as measured by dual x-ray absorptiometry. This low BMD was correlated with increase serum C-telopeptide type I collagen, a marker of bone resorption. Thus, HIV-infected persons possess a significant risk to develop reduced bone mineral density due to increase bone resorption activity prior to treatment. Further studies are needed to evaluate the association of disease severity and bone resorption markers.

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