SciForschen
Open HUB for Scientific Research
Journal of Drug Research and Development
ISSN 2470-1009 | Open Access

RESEARCH ARTICLE
Volume 4 - Issue 1 | DOI: http://dx.doi.org/10.16966/2470-1009.139

Assessment of Plasma Levels of Bone Metabolism Biomarkers among HIV Infected Adult Patients in Ethiopia

Kissi Mudie*, Bikila Negassa, Feyissa Challa, Meron Sileshi, Tigist Getahun, Abenezer Ayalkibet, Tadesse Lejisa, Demirew Bikila, Yosef Tolcha, Wossen Habtu, Zeleke Geto, Atsbeha G/Igzabixier, Genet Ashebir and Tirhas H/Kiros

Ethiopian Public Health Institute (EPHI), Addis Ababa, Ethiopia

***Corresponding author:** Kissi Mudie, Ethiopian Public Health Institute (EPHI), Addis Ababa, Ethiopia, **E-mail:** kissimudiey@yahoo.com

Received: 07 Dec, 2017 | **Accepted:** 08 Feb, 2018 | **Published:** 15 Feb, 2018

Citation: Mudie K, Negassa B, Challa F, Sileshi M, Getahun T, et al. (2018) Assessment of Plasma Levels of Bone Metabolism Biomarkers among HIV Infected Adult Patients in Ethiopia. J Drug Res Dev 4(1): dx.doi.org/10.16966/2470-1009.139

Abstract

Background: Bone diseases are common complications in HIV infected patients with increased fracture rates. Factors, calcium and Vitamin D deficiency, depression and smoking are contributed to increased prevalence of this disease. Some studies reported that antiretroviral therapy may also relate to bone diseases in HIV patients.

Aim of the study: To assess plasma levels of bone metabolism biomarkers in HIV infected patients before and after ART treatment.

Materials and methods: Leftover blood samples from advanced clinical monitoring project were used for this study. 156 individuals with HIV infection before and after ART at different stages of treatment (0, 6 and 12 months) were investigated. Plasma level of bone metabolism biomarkers like PTH, phosphate, Vitamin D and Osteocalcin were measured using Cobas 6000 chemistry machine. Data analysis was carried out by means of statistical package for social science version 16.

Results: The mean level of PTH and Osteocalcin increased significantly in HIV infected patients taking ART compared to HIV patients not taking ART (p value<0.05). Viral load was negatively correlated with PTH, Osteocalcin, Vitamin D and Phosphate.

Conclusions: As the duration of treatment with ART extended, further increase of the higher rate of bone turnover in HIV-infected patients was observed.

Keywords: HIV; ART; Bone; Biomarkers

Background

Bone metabolism

Bone is a specialized connective tissue made up of glycoproteins, proteoglycans and collagen impregnated with mineral, hydroxyapatite [1].

Bone constantly being repaired and remodeled throughout an individual's lifetime [2]. This continuous remodelling undergoes through bone formation and bone resorption. These two processes are tightly coupled to each other, so that the amount of bone formation equals the amount of newly formed bone. This balance is regulated through the action of various hormones like Parathyroid Hormone (PTH), Vitamin D, and other steroid hormones and local mediators [3].

Bone formation is favored in healthy growing children; whereas bone resorption is slightly higher as age advanced. However, in healthy adults, bone remodeling is balanced. Bone mineral density increases during childhood and adolescence, reaches peak values in adult life and declines with age thereafter [4,5]. Other confounding factors like ageing, metabolic bone diseases, therapeutic interventions, hyperthyroidism, hematologic disorders, genetic factors, gastrointestinal diseases, rheumatoid arthritis, hypogonadism, depression, alcoholism, renal failure, cardiac failure, deficiency of calcium and Vitamin D and smoking are characterized by imbalances in bone turnover [3,6].

Bone metabolic disorder in HIV patients

Bone diseases are common complications in HIV infected patients with fracture rates three times higher than HIV uninfected individuals [7-9].

Bone loss in HIV infected persons can be aggravated by pathophysiological disruptions in the body such as hormonal imbalances, inflammatory cytokines action and kidney pathological processes [10]. Other studies revealed that bone loss in HIV infected individuals occurs as a consequence of HIV viral protein and antiretroviral therapy [11-18].

Antiretroviral drugs and bone metabolic complications

Antiretroviral Drugs (ART) are medication for treatment of infection by HIV. These drugs block various steps of the HIV replication cycle and inhibit the progression of the virus [19].

ART has significantly improved the morbidity and mortality in HIV-infected patients by preventing opportunistic infections, restoring and retaining immune function, increasing CD4+ cell counts, and delaying progression to AIDS [20]. These benefits are compromised by numerous side effects, adverse clinical events and toxicities [21].

Due to complex metabolic complications of HIV infection and its treatment, the reduction in bone mineralization was observed in a large percentage of HIV-infected patients. This might be as the result of interplay of host, viral and specific antiretroviral factors [22].

One longitudinal cohort study revealed a marked incidence of low BMD in a large number of patients with long-term HIV infection and prolonged antiretroviral therapy [22].

Bone metabolism biomarkers

Bone is made of collagen, Calcium and phosphate [23]. Parathyroid Hormone (PTH) is a polypeptide hormone which regulates plasma Ca^{2+} and PO_4 [24-26] by sensing protein in the cell membrane of the parathyroid cells [27,28].

Osteocalcin is the main non-collagen protein of bone matrix which is synthesized during the bone formation [7,29]. The deficiencies of calcium and phosphate lower the formation of the hydroxyapatite crystals, which make the free osteocalcin to circulate in the blood. This results in increased concentrations of osteocalcin in the plasma of osteoporotic patients [8].

Due to its tissue specificity; its wide availability and its relatively low variation within person, osteocalcin can be considered as a clinical index of bone turnover. In general, a high bone turnover rate can be characterized by elevated levels of plasma osteocalcin [29].

Bone mineralization and calcium homeostasis can be maintained by the action of Vitamin D. Low Vitamin D concentrations might lead to secondary hyperparathyroidism that results in bone resorption and fracture risk [30].

Statement of Problem

Though, the introduction of potent antiretroviral drugs resulted in significant reduction of mortality and morbidity in HIV patients, the new problems due to HIV infection and adverse effects of antiretroviral drugs have been critical [31]. Currently, bone metabolic disorders have been emerged as a worrisome complication in HIV infected patients. It is not clear that if HIV infection itself or antiretroviral treatment or both are causes of bone loss; however, most of the studies have shown a high prevalence of osteoporosis in HIV/AIDS patients [32]. In this area, no studies were conducted in Ethiopia and this study supports to understand the relation between bone density abnormalities and patients infected with HIV before and after treatment; subsequently to identify and monitor the complications caused by bone disease.

The objective of this study was to assess plasma levels of bone metabolism biomarkers in HIV patients before and after treatment.

Material and Methods

Study design

This study was a retrospective cohort study type.

Study population

The study was conducted using left over samples collected from 7 university hospitals for the project of Advanced Clinical Monitoring (ACM) of ART in Ethiopia. These university hospitals were Addis Ababa University, Jimma University, Haromaya University, Mekelle University, Gondar University, Hawassa University and Armed forces general hospital. ACM samples from university hospitals were selected because these hospitals has begun providing free ART under the national program and were treating one of every eight ART patients in the country. These institutions were also participating in the overall roadmaps for ART rollout laid out by Ministry of Health and their regional health bureaus.

Data was collected retrospectively from database. Baseline data including clinical history, CD4 cell count, viral load and other clinical examinations assessed during follow up were considered. Participants who have been diagnosed with disease affecting bone mineral density like TB, chronic kidney diseases and hyperthyroidism were excluded. Chronic smokers, alcohol and drug users were also excluded from this study.

Sample size

A total of 156 samples of adult HIV patients before and after ART were used. HIV patients who did not take the treatment were used as a baseline test group. HIV patients under ART at different time intervals (0, 6 and 12) were investigated. All groups were gender and age matched.

Demographic characteristics

Out of 156 study participants, 45.5% were female. All study participants were in the age group between 18-45 years.

Specimen collection, handling, storage and processing

Plasma samples stored at -80°C was used for this study. The samples were de-frozen and centrifuged at 3000 rpm for 10 minutes before analysis.

Laboratory sample analysis

Blood levels of bone turnover biomarkers including Osteocalcin (OC), Phosphate, intact Parathyroid hormone (iPTH) and Vitamin D were determined using standard procedures [33,34] by Cobas 6000 chemistry analyzer. Quality controls and calibrators were run before sample analysis to confirm the quality of the testing reagents and proper functioning of the machine.

Statistical analysis

Statistical analysis was performed using statistical package for windows (SPSS v16). The data were entered into the SPSS format and the continuous variables were summarized by using the mean and standard error of the mean. The data were analyzed by one way ANOVA followed by Tukey-Kramer post hoc test. P values less than 0.05 were considered to be statistically significant. Pearson's correlation test was also performed to compare the bone metabolism biomarkers with CD4 cell count and viral load.

Results

Determination of bone biomarkers

The mean level of iPTH and Osteocalcin increased significantly in HIV infected patients taking ART compared to HIV patients not taking ART (p value<0.05). There were no significant differences in plasma levels of phosphorous and Vitamin D in HIV patients taking ART and not taking ART (p value>0.05) (Table 1).

There was no significant variation between male and females with HIV before and after treatment in plasma levels of iPTH, Osteocalcin and phosphate. However; there is a significant difference in plasma level of Vitamin D (Table 2).

The results of the Pearson's correlation test showed CD4 cell count was positively correlated with Osteocalcin and negatively correlated with iPTH, Vitamin D and phosphate (Table 3). Viral load was negatively correlated with plasma levels of iPTH, Osteocalcin, Vitamin D and phosphate (Table 4).

Table 1: Comparison of the Mean ± SEM of the bone biomarker parameters

Parameter	0 month	6 months	12 months
iPTH (ng/dL)	21.84 ± 2.04	32.21 ± 2.60**	37.36 ± 5.30**
Osteocalcin (ng/mL)	15.52 ± 1.03	28.33 ± 1.77**	31.08 ± 3.25**
Phosphate (mmol/L)	1.23 ± 0.02	1.19 ± 0.03	1.15 ± 0.04
VITD (ng/mL)	20.21 ± 1.28	22.13 ± 1.44	21.08 ± 0.83

SEM: Standard Error of the Mean; iPTH: intact Parathyroid Hormone; VITD: Vitamin D; **: Highly Significant

Table 2: Comparison of plasma levels of PTH, calcium, phosphorous and Alkaline Phosphatase between male and females with HIV (mean ± SEM)

Parameter	Male HIV patients (n=85)	Female HIV patients (n=71)	P value
iPTH (ng/dL)	28.91 ± 2.13	28.34 ± 2.82	0.87
Osteocalcin (ng/mL)	21.74 ± 1.43	22.89 ± 1.75	0.61
Phosphate (mmol/L)	1.18 ± 0.02	1.23 ± 0.03	0.16
VITD (ng/mL)	23.52 ± 1.10	18.13 ± 1.19	0.001

SEM: Standard Error of the Mean; iPTH: intact Parathyroid Hormone; VITD: Vitamin

Table 3: Bivariate correlation between CD4 and variables

Parameter		CD4	iPTH	Osteo	Phos	VITD
CD4	Pearson Correlation	1	-0.125	0.123	-0.132	-0.094
	Sig. (2-tailed)		0.233	0.250	0.190	0.358
	N	100	92	89	100	98

iPTH: intact Parathyroid Hormone; Osteo: Osteocalcin; Phos: Phosphate; VITD: Vitamin D; Sig: Significant; N: Number of participants

Table 4: Bivariate correlation between viral load and variables

Parameter		Viral Load	iPTH	Osteo	Phos	VITD
Viral Load	Pearson Correlation	1	-0.013	-0.119	-0.052	-0.078
	Sig. (2-tailed)		0.916	0.308	0.644	0.481
	N	83	73	75	83	83

iPTH: intact Parathyroid Hormone; Osteo: Osteocalcin; Phos: Phosphate; VITD: Vitamin D; Sig: Significant; N: Number of participants

Discussion

In this study prolonged exposure to antiretroviral drugs resulted in increased bone resorption of HIV patients. This finding aligned with the study of Anna et al. [21] that indicated around 75% of study participants under long follow-up of ART treatment has shown some grade of low bone mass, followed by one third having developed osteoporosis.

In the current study, bone loss caused by ART is evident by significant increase in the mean level of PTH in HIV infected patients taking ART compared to HIV patients not taking ART. This finding is in agreement with the work done by Zhao et al. [35] that indicated increased plasma PTH level caused an increase in bone resorption. Study done by Bonofiglio et al. [36] also stated that PTH is inversely related to bone mineral density after adjustment for calcium intake and bone age.

According to Kota et al. [37] people with hyperparathyroidism produce too much parathyroid hormone for too long. This can cause calcium to be dissolved from the bones. Over time, if the hyperparathyroidism is not treated, loss of calcium from the bones can lead to decreased bone density and weakening of the bones. Eventually, osteoporosis may develop.

According to our finding significant increase in the mean level of Osteocalcin was also observed in HIV infected patients taking ART compared to HIV patients not taking ART. This is in consistent with the work done by Deeks et al. [38] that indicated ART is accompanied by increases in bone biomarker activation, which may lead to mechanisms underlying bone loss in HIV infected individuals.

Our study observed that the mean levels of Vitamin D was <30 ng/ml in all groups. According to Sahota et al. [39], the lower levels of Vitamin D (<30 ng/ml) and increasing PTH levels were associated with lower bone mineral density. This

leads to the suggestion that secondary hyperparathyroidism can be the mechanism whereby Vitamin D deficiency that could contribute to bone loss.

This study showed significant decrease in mean levels of Vitamin D in female when compared to male. This finding is in agreement with study done by Verdoia et al. [40], where female gender was associated with lower Vitamin D levels.

Conclusions

As the duration of treatment with ART extended, further increase of the higher rate of bone turnover in HIV-infected patients was observed.

Recommendation

Assessment, screening, diagnosis and follow-up of bone turnover should be conducted in all HIV patients commencing ART; throughout the course of ART therapy. Moreover; specific ART drug responsible for osteoporosis should be identified and remedial action should be taken accordingly.

Limitation of the Study

This study didn't show Calcium pattern because blood Plasma samples taken in EDTA tubes were used that is Calcium can be masked by plasma EDTA.

Ethical Approval

The study was conducted following the ethical approval by Scientific and Ethical Research Office (SERO), Ethiopian Public Health Institute (SERO-031-12-2016).

Competing Interests

The authors have nothing to declare with regards to this study.

Acknowledgements

The authors are thankful to HIV team specially Dr. Desta Kassa, Saro Abdella and Rahel Tilahun of Ethiopian Public Health Institute for providing leftover stored samples and to Mr. Dawit for his help with providing and cleaning of the data prior to data analysis. We would like to thank also Mr. Girum Taye for his support during statistical data analysis.

References

1. Wheater G, Elshahaly M, Tuck SP, Datta HK, van Laar JM (2013) The clinical utility of bone marker measurements in osteoporosis. J Transl Med 11: 201.
2. Kanis JA (2002) Diagnosis of osteoporosis and assessment of fracture risk. Lancet 359: 1929-1936.
3. Markus JS (2005) Biochemical Markers of Bone Turnover Part I: Biochemistry and Variability. Clin Biochem Rev 26: 97-122.
4. Marks SC, Odgren PR (2002) Structure and Development of the Skeleton. In: Bilezikian JP, Raisz LG, Rodan GA (eds) Principles of Bone Biology. 2nd Edition, Elsevier.
5. Mora S, Sala N, Bricalli D, Zuin G, Chiumello G, et al. (2001) Bone mineral loss through increased bone turnover in HIV-infected children treated with highly active antiretroviral therapy. AIDS 15: 1823-1829.
6. Bonjoch A, Figueras M, Estany C, Perez-Alvarez N, Rosales J, et al. (2010) High prevalence of and progression to low bone Mineral density in HIV-infected patients: a longitudinal cohort study. AIDS 24: 2827-2833.
7. Badie BM, Soori T, Kheirandish P, Izadyar S, SeyedAlinagh S, et al. (2011) Evaluation of Bone mineral density in Iranian HIV/AIDS Patients. Acta Med Iran 49: 460-467.
8. Marco B, Davide G, Fabio V, Elisa DC, Laura C, et al. (2009) Metabolic bone disease in HIV infection. AIDS 23: 1297-1310.
9. Triant VA, Brown TT, Lee H, Grinspoon SK (2008) Fracture prevalence among human immunodeficiency virus (HIV)-infected versus non-HIV-infected patients in a large U.S. healthcare system. J Clin Endocrinol Metab 93: 3499-3504.
10. Brown TT, Qaqish RB (2006) Antiretroviral therapy and the prevalence of osteopenia and osteoporosis: a meta-analytic review. AIDS 20: 2165-2174.
11. Vikulina T, Fan X, Yamaguchi M, Roser-Page S, Zayzafoon M, et al. (2010) Alterations in the immuno-skeletal interface drive bone destruction in HIV-1 transgenic rats. Proc Natl Acad Sci U S A 107: 13848-13853.
12. Mayer KH, Amorosa V, Tebas P (2006) Bone disease and HIV infection. Clin Infect Dis 42: 108-114.
13. Haskelberg H, Carr A, Emery S (2011) Bone turnover markers in HIV disease. AIDS Rev 13: 240-250.
14. Mondy K, Yarasheski K, Powderly WG, Whyte M, Claxton S, et al. (2003) Longitudinal evolution of bone mineral density and bone markers in human immunodeficiency virus-infected individuals. Clin Infect Dis 36: 482-490.
15. Ofotokun I, Weitzmann MN (2010) HIV-1 infection and antiretroviral therapies: risk factors for osteoporosis and bone fracture. Curr Opin Endocrinol Diabetes Obes 17: 523-529.
16. Powderly WG (2012) Osteoporosis and bone health in HIV. Curr HIV/AIDS Rep 9: 218-222.
17. Rothman MS, Bessesen MT (2012) HIV infection and osteoporosis: pathophysiology, diagnosis, and treatment options. Curr Osteoporos Rep 10: 270-277.
18. Saccomanno MF, Ammassari A (2011) Bone disease in HIV infection. Clin Cases Miner Bone Metab 8: 33-36.
19. Ofotokun I, McIntosh E, Weitzmann MN (2012) HIV: inflammation and bone. Curr HIV/AIDS Rep 9: 16-25.
20. Michaud V, Bar-Magen T, Turgeon J, Flockhart D, Desta Z, et al. (2012) The Dual Role of Pharmacogenetics in HIV Treatment: Mutations and Polymorphisms Regulating Antiretroviral Drug Resistance and Disposition. Pharmacol Rev 64: 803-833.
21. Awodele O, Agbaje EO, Adesina EA, Akintonwa A (2011) Hepatoprotective Role of Neutrosec on Hepatic Damage Induced by Combination of Zidovudine and Combined Anti-tuberculous Agents in Rats. Tokai J Exp Clin Med 36: 31-36.

22. Mudie K, Seifu D, Challa F, Abebe A, Debella A, et al. (2014) Hepatoprotective activity of aqueous seed extract of *Nigella sativa* against highly active antiretroviral therapy induced hepatotoxicity in rats. Pharmacology OnLine 3: 11-21.

23. Rabenda V, Bruyère O, Reginster JY (2011) Relationship between bone mineral density changes and risk of fractures among patients receiving calcium with or without Vitamin D supplementation: a meta-regression. Osteoporos Int 22: 893-901.

24. Delmas PD, Meunier PJ (1997) The management of Paget's disease of bone. N Engl J Med 336: 558-566.

25. Kumar R, Thompson JR (2011) The regulation of parathyroid hormone secretion and synthesis. J Am Soc Nephrol 22: 216-224.

26. Brown EM (1982) PTH secretion *in vivo* and *in vitro*. Regulation by calcium and other secretagogues. Miner Electrolyte Metab 8: 130-150.

27. Brown EM, Hebert SC (1997) Calcium-receptor regulated parathyroid and renal function. Bone 20: 303-309.

28. Brown EM, Hebert SC (1996) A cloned extracellular Ca($^{2+}$)-sensing receptor: molecular mediator of the actions of extracellular Ca^{2+} on parathyroid and kidney cells? Kidney Int 49: 1042-1046.

29. Cepelak I, Cvoriscec D (2009) Biochemical markers of bone remodeling-review. Biochemia Medica 19: 17-35.

30. Madar AA, Knutsen KV, Stene LC, Brekke M, Lagerløv P, et al. (2015) Effect of Vitamin D3-supplementation on bone markers (serum P1NP and CTX): A randomized, double blinded, placebo controlled trial among healthy immigrants living in Norway. Bone Rep 2: 82-88.

31. Kayode AAA, Kayode OT, Aroyeun OA, Stephen MC (2011) Hematologic and Hepatic Enzyme Alterations Associated with Acute Administration of Antiretroviral Drugs. J Toxicol Pharmacol 6: 293-302.

32. Haifa MM, Reel MM, GadAllah M, Nassr Eldin MA (2014) Assessment of serum levels of bone metabolism biomarkers in Sudanese with HIV infection. J Appl Med Sci 2: 3270-3273.

33. Tietz NW (1999) In: Burtis CA, Ashwood ER, Tietz NW (eds) Tietz Text book of Clinical Chemistry. 3rd edition, WB Saunders, Philadelphia.

34. Thomas L (1998) Clinical Laboratory Diagnostics: Use and assessment of Clinical Laboratory Results. 1st edition, TH-Books.

35. Zhao LJ, Liu YJ, Liu PY, Hamilton J, Recker RR, et al. (2007) Relationship of obesity with osteoporosis. J Clin Endocrinol Metab 92: 1640-1646.

36. Bonofiglio D, Maggiolini M, Catalano S, Marsico S, Aquila S, et al. (2001) Bone mineral density is inversely related to parathyroid hormone in adolescent girls. Horm Metab Res 33: 170-174.

37. Kota S, Jammula S, Kota S, Meher L, Modi K (2013) Correlation of Vitamin D, bone mineral density and parathyroid hormone levels in adults with low bone density. Indian J Orthop 47: 402-407.

38. Deeks SG (2009) Immune dysfunction, inflammation, and accelerated aging in patients on antiretroviral therapy. Top HIV Med 17: 118-123.

39. Sahota O, Mundey MK, San P, Godber IM, Lawson N, et al. (2004) The relationship between Vitamin D and parathyroid hormone: Calcium homeostasis, bone turnover, and bone mineral density in postmenopausal women with established osteoporosis. Bone 35: 312-319.

40. Verdoia M, Schaffer A, Barbieri L, Di Giovine G, Marino P, et al. (2015) Impact of gender difference on Vitamin D status and its relationship with the extent of coronary artery disease. Nutr Metab Cardiovasc Dis 25: 464-470.