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Bone Health and Impact of Tenofovir Treatment in Men with Hepatitis-B Related Chronic Liver Disease

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Background: **Chronic liver disease (CLD) has been shown to have an adverse impact on bone health. Hepatitis-B related CLD and its treatment with tenofovir may have additional effects on skeleton.** ***Objective:*** **To study the impact of HBV related CLD and its treatment with Tenofovir on bone health in Indian subjects.** ***Methods:*** **This cross sectional study included men (18–60 years) and comprised of three groups: Group-1 was treatment naïve HBV related CLD (n = 79), Group-2 those with HBV related CLD on tenofovir for at least 1 year (n = 136), Group-3 age, sex and body mass index (BMI) matched healthy controls (n = 58). Bone biochemistry and bone mineral density (BMD) at spine, femoral neck (FN) and forearm were studied. Independent t-test or ANOVA was used to compare the means of continuous variables and chi-square test for categorical variables. Multiple logistic regression was used to assess the factors causing low bone mass (LBM) at FN.** ***Results:*** **A significantly greater proportion (P < 0.05) of patients (40%) with CLD (group 1 and group 2) had vitamin D deficiency (<20 ng/mL) in comparison with control group (22%). The mean serum C-Terminal telopeptide was significantly higher (P < 0.05) and the mean BMD was significantly lower (P < 0.05) in subjects with HBV related CLD than controls. The prevalence of LBM was higher in group 1 at the spine (31%) and forearm (18.4%) when compared to controls (8.1% and 7.8% respectively) (P < 0.05). The proportion of patients with LBM at FN was highest in group 2 (12.3%) compared to those in group 1 (8%) and group 3 (4%) (P < 0.05). Advanced age, low BMI, and high viral load (>10,000 IU/mL) emerged as significant risk factors for LBM at FN.** ***Conclusion:*** **The impact of hepatitis-B related CLD as well as its treatment on bone health is significant. Bone health need to be periodically evaluated in these subjects especially in older men who are lean and have a higher viral load. (J Clin Exp Hepatol 2017;xx:1–5)**

Chronic liver disease (CLD) has been shown to have an adverse impact on bone health. Hepatic osteodystrophy is a term that not only refers to osteomalacia but also to the low bone mass that is seen in patients with CLD. This entity is clinically relevant, as it has been shown to increase the risk of spine fractures with its associated increased morbidity.[1] The clinical scenario in patients with chronic hepatitis-B associated osteodystrophy is even more complex as herein, not only the etiology for hepatic osteodystrophy is multifactorial but even the therapy of infection could seem to alter the bone health.[2] Poor nutrition, low body mass index, hypogonadism, sedentary life style, elevated inflammatory cytokines, low Insulin-like growth factor-1, unconjugated hyperbilirubinemia and suboptimal vitamin-K are some of the biological factors associated with low bone mass in patients with hepatitis-B virus associated (HBV) CLD.[3–6]

Several studies have shown a higher prevalence of low bone mass in patients with CLD. However, the subjects with CLD secondary to hepatitis-B form a unique cohort of patients, especially in India, where the diagnosis occurs very late.[7] There are limited data with regards to bone health in hepatitis-B related CLD in the Indian setting. We aimed to study the prevalence of vitamin-D deficiency and low bone mass in patients with CLD secondary to hepatitis-B infection and also to ascertain the factors that were associated with low bone mass at femoral neck (FN). The effect on FN was especially chosen to be studied, since fracture secondary to low bone mass in this region has been shown to be associated with significant morbidity and mortality. We also sought to look at the impact of treatment with tenofovir on bone health in these subjects.

Keywords: chronic liver disease, bone mineral density, hepatitis-B, tenofovir

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Abbreviations: BMI: body mass index; CLD: chronic liver disease; FN: femoral neck; LBM: low bone mass

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METHODS

This was a cross sectional study (n = 273) conducted at a south Indian tertiary care institution between January 2014 and December 2015 after an approval by the institutional review board. Study subjects included men between the age of 18 to 60 years and comprised three groups: Group-1 included those with established HBV related CLD but were treatment naïve (n = 79), Group-2 included those with HBV related CLD on Tenofovir for at least 1 year duration (n = 136), Group-3 included normal age, sex and body mass index (BMI) matched healthy controls who were recruited from the community (n = 58). CLD was diagnosed based on clinical, laboratory, radiological and endoscopic criteria. We excluded patients with nonhepatitis-B related CLD, hepatocellular carcinoma, current alcohol consumption, previous fractures, secondary osteoporosis, and other systemic illnesses and those on bone active medications.

Data regarding age, body mass index, hepatitis-B viral load, insulin-like growth factor-1, testosterone, bone mineral parameters, bone mineral density (BMD) and bone resorption marker—C-terminal telopeptide of type-1 collagen (CTX) were collected and systematically analyzed.

An overnight fasting blood sample was obtained for the estimation of serum calcium (8.6–10.2 mg/dL), phosphate (2.5–5 mg/dL), albumin (3.5–5.0gm/dL), alkaline phosphatase (40–125 IU/L), creatinine (0.6–1.2 mg/dL), 25-hydroxy vitamin D (30–75 ng/mL) hereafter referred to as vitamin D, intact parathyroid hormone (iPTH) (8–50 pg/mL), CTX (100–584 pg/ml) and Insulin like growth factor-1 (IGF-1) (age and sex matched reference changes). These reference ranges are as provided by the institutional laboratory. Vitamin D deficiency was defined as a vitamin D level of less than 20 ng/mL.[9] The vitamin D, intact PTH, and IGF-1 were measured by chemiluminescence method using Immulite analyzer 2000. Serum CTX was measured using electro-chemiluminesence using Roche Elecsys Modular E170 analyzer. Other biochemical parameters were measured in a fully automated and computerized micro-analyzer (Hitachi Roche Modular P 800 model). The intra-assay and inter assay coefficients of variation for these analytes were 1 to 5%.

BMD was assessed using the Hologic DXA QDR 4500 Discovery A machine at the lumbar spine, distal forearm and FN by the same technician. Precision was 2 percent at all the three measured sites. The WHO classification was used for categorization of BMD. Low bone mass was defined as a Z score of less than minus 2 (<2 SD below the mean of age-matched reference data).

SPSS V.16.0 software (IBM, Corp, USA) was used for data analysis. Independent t-test was used to compare the means of two continuous variables as they were normally distributed. One way analysis of variance (ANOVA) was used to calculate significance among 3 groups as they were normally distributed. Categorical variables were analyzed with the chi square test. Univariate regression analysis was done to assess the effect of individual risk factors on FN resulting in low bone mass. The factors which emerged as significant (P-value $\leq$ 0.10) in univariate analysis were further analyzed by multiple logistic regression. Odds ratio was calculated and was considered to be significant when P-value was $\leq$ 0.05.

RESULTS

The demographic data and baseline biochemical parameters among the 3 groups are summarized in Table 1. The mean age and BMI were similar in all 3 groups. The baseline biochemistry was similar except for serum CTX which was found to be significantly higher in patients with HBV related CLD than controls. The median viral load was 10,000 IU/mL. Forty percent of patients with CLD (31% in Group-1 and 43% in Group-2) had vitamin D deficiency compared to 22% among the controls ($P \leq 0.05$), although mean 25-hydroxy vitamin-D level did not differ among 3 groups. Mean IGF-1 was low in HBV infected individuals (Group-1 and 2) when compared to Group-3 (Table 1).

The mean BMD at all three sites was significantly lower ($P < 0.05$) in subjects with HBV related CLD (group 1 and 2) than in age, sex and BMI matched controls (Table 2). The prevalence of low bone mass was higher in group 1 at the spine (31%) and distal forearm (18.4%) when compared to controls (8.1% and 7.8% respectively) ($P < 0.05$). The proportion of patients with low bone mass at FN was higher in group 2 (12.3%) compared to those in group 1 (8%) and group 3 (4%) ($P < 0.05$).

We also studied the impact of high viral load (>10,000 IU/mL) on BMD. Significantly more number of patients with high viral load had a lower bone mass at FN as compared to those with viral load <10,000 IU/mL. This trend was also noted at spine and distal forearm, however statistical significance was not achieved at these sites (Table 3).

Multiple logistic regression analysis to assess the factors associated with low bone mass at FN in patients with hepatitis-B related CLD revealed advancing age, low BMI and a high viral load (>10,000 IU/mL) as the significant risk factors influencing BMD at FN (Table 4).

DISCUSSION

This study highlights the impact of hepatitis-B related CLD and its treatment with tenofovir on skeletal health. In addition, we also assessed key factors affecting bone mass at FN in these subjects. Two-fifth of the subjects with hepatitis-B had vitamin D deficiency. A significantly higher bone resorption (serum CTX), a lower mean BMD and a higher prevalence of low bone mass was found in subjects

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Table 1 Demography and Biochemistry Among the 3 Groups.

Parameters	Chronic liver disease not on Tenofovir (*n* = 79) Group-1 Mean ± SD	Chronic liver disease on Tenofovir (*n* = 136) Group-2 Mean ± SD	Controls (*n* = 58) Group-3 Mean ± SD	ANOVA *P* value	*t*-Test *P* value
Age (years)	40.5 ± 10	38.4 ± 10.8	38 ± 6.2	0.29	–
Body mass index (kg/m^2)	23.4 ± 3.5	23.3 ± 3.7	24.2 ± 4.5	0.30	–
Calcium (mg/dL)	8.9 ± 0.06	8.8 ± 0.5	8.7 ± 0.46	0.19	–
Phosphorus (mg/dL)	3.4 ± 0.9	3.3 ± 0.6	3.6 ± 0.4	0.11	–
Parathormone (pg/ml)	51.4 ± 35	52.6 ± 34.1	41.6 ± 15.4	0.098	–
25 (OH) Vitamin D (ng/ml)	23.2 ± 12.6	24.5 ± 11.3	26.8 ± 8.7	0.12	–
CTX (ng/ml)	700.9 ± 334	650.4 ± 327.1	520.1 ± 179.6	0.03	0.280 (1vs2) 0.004 (2vs3) 0.000 (1vs3)
IGF-1 (ng/ml)	120 ± 68	109 ± 61.6	180 ± 24.1	0.051	0.225 (1vs2) 0.000 (2vs3) 0.000 (1vs3)
Testosterone (ng/ml)	492.6 ± 288.6	493.7 ± 200.2	446.7 ± 183	0.14	–
Alk Phos (U/L)	103.84 ± 73.86	110.63 ± 83.75	71.82 ± 16.12	0.093	–

with chronic hepatitis-B infection. Though the spine and forearm were most affected in treatment naïve hepatitis-B patients, the FN was more involved in tenofovir treated patients. Advancing age, low BMI and a high viral load (>10,000) emerged as the most pertinent risk factors for low bone mass at FN in these individuals.

A higher proportion of subjects with HBV infection was found to have vitamin D deficiency as compared to controls. Low 25 (OH) vitamin D has been reported in patients with CLD of any etiology, probably due to limited exposure to sunlight and poor nutrition of these subjects in view of their primary illness.[2,8] In addition, factors such as intestinal malabsorption, poor entero-hepatic circulation, and low vitamin D synthesis in skin affected with excess bilirubin could contribute to low vitamin D levels in these individuals.[9]

Bone resorption marker serum C-terminal telopeptide was found to be significantly higher in patients with CLD as compared to controls. Urtemen et al. found significantly higher level of bone resorption markers and lower bone formation markers among patients with CLD compared to healthy controls.[10] However, there is no previously published literature on bone turnover markers specifically in patients with hepatitis-B related CLD.

Lumbar spine was the most commonly affected site with low bone mass in our patients with treatment naïve HBV related CLD which has also been demonstrated previously. However, when patients are exposed to tenofovir (>1 year), consistently FN seems to be more affected.[11,12] Reports of hypophosphatemia mediated by tenofovir are often cited to be secondary to elevated FGF-23 levels.[14] Data from HIV-infected individuals suggest that tenofovir

Table 2 Comparison of Mean BMD at Different Sites Among the 3 Groups (ANOVA).

Bone mineral density	Chronic liver disease not on Tenofovir Group 1 (*n* = 79) Mean ± SD	Chronic liver disease on Tenofovir Group 2 (*n* = 136) Mean ± SD	Controls Group 3 (*n* = 58) Mean ± SD	*P* value (*t*-test)	*P* value (ANOVA)
Forearm (gm/cm^2)	0.603 ± 0.084	0.619 ± 0.067	0.650 ± 0.046	0.126 (1 vs 2) 0.001 (2 vs 3) 0.000 (1 vs 3)	0.001
Lumbar spine (gm/cm^2)	0.903 ± 0.142	0.940 ± 0.126	0.973 ± 0.118	0.090 (1 vs 2) 0.090 (2 vs 3) 0.002 (1 vs 3)	0.009
Femoral neck (gm/cm^2)	0.771 ± 0.115	0.741 ± 0.138	0.835 ± 0.238	0.104 (1 vs 2) 0.000 (2 vs 3) 0.003 (1 vs 3)	0.010

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Table 3 Low Bone Mass in Patients With Different Viral Load (Chi-square Test).

Bone mineral density sites	Prevalence of low bone mass	
	Viral load <10,000 IU/ml *n* = 120	Viral load >10,000 IU/ml *n* = 95
Spine *n* (%)	29 (24.1)	23 (24.2)
Femoral neck *n* (%)*	6 (5)	13 (13.6)*
Distal Forearm *n* (%)	18 (15)	19 (20)

*$P < 0.05$.

Table 4 Multiple Logistic Regression Analysis: Factors Causing Low Bone Mass at Femoral Neck in Patients With Hepatitis B Related CLD.

Parameters studied	Odds (C.I)	*P* Value
Viral load (>10,000)	2 (1.1–18.5)	0.05
BMI (<23 kg/m^2)	8 (1.3–26.8)	0.022
Testosterone (<300 ng/dl)	12 (0.3–120)	0.561
IGF-1 (ng/ml)	3 (0.2–144)	0.471
Vitamin D deficiency (<20 ng/ml)	6 (0.4–36)	0.851
Age (>40) in years	4 (1.2–21)	0.026

use is associated with Fanconi syndrome and a greater risk of hip fractures.[15] Moreover this effect of tenofovir is often reversible and the hypophosphatemic osteomalacia is known to recover over a period of 6 months.[13,14]

Poor nutritional calcium intake, low body mass index, hypogonadism, sedentary life style, elevated inflammatory cytokines, low Insulin like growth factor-1, unconjugated hyperbilirubinemia, and suboptimal vitamin K are some of the biological factors associated with low bone mass in patients with hepatitis-B virus associated (HBV) CLD.[3–6] Tenofovir induced bone loss is mainly attributed to its ability to alter mitochondrial function and gene expression by its preferential uptake in osteoclasts and osteoblasts.[11] In addition, tenofovir is also known to cause proximal renal tubular dysfunction which may have an adverse impact on bone mineralization.[12] The plausible reason for this differential action of tenofovir on FN is not well established.[13] A higher plasma vitamin D binding protein and subsequently a lower free calcitriol level is described in patients treated with Tenofovir.[16]

There is conflicting evidence on the association of higher viral load with low bone mass in patients with hepatitis C, more so information with regards to impact of viral load in HBV related CLD on BMD is even more limited.[17–19] Biological factors and certain candidate genes have been shown to be associated with osteoporosis in other studies with CLD, but are not clearly defined and are poorly understood.[20] In addition, increased osteoclast activity is mediated through osteoclastogenic proinflammatory cytokines such as interleukin 1 and tumor necrosis factor, which probably explains the link between the viral load and low bone mass.[21]

Other factors that could be associated with low bone mass in this setting include lower vitamin K levels which are essential for the synthesis of osteocalcin and bone matrix protein.[21,22] IGF-1 deficiency has also been cited to affect osteoblast differentiation and proliferation. These levels are consistently found low in patients with CLD as is also noted in our study.[9,23] Hypogonadism associated with CLD is known to affect bone mass especially in women with lower estradiol levels, however in men with CLD despite having slightly higher estradiol levels, there is no protective effect seen.[24] In our study, these factors did show a trend but did not attain statistical significance and needs to be validated in larger studies. Thus hepatitis-B related bone disease is multifactorial with both conventional and hepatitis-B specific risk factors contributing to low bone mass in these individuals.[25]

STRENGTHS AND LIMITATIONS OF THE STUDY

This is the first paper from the Indian subcontinent to study the impact of hepatitis B related CLD and tenofovir on bone health. This being a cross-sectional study may not truly reflect the changes in skeletal system occurring over a period of time especially because comparison of bone health before and after treatment with tenofovir was not performed. In addition, risk factors such as poor physical activity, nutritional status, socio-economic status, smoking, vitamin-K which could influence BMD were not studied in subjects.

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CONCLUSION

The impact of hepatitis-B related CLD on bone health is significant. Bone health need to be actively assessed in patients with chronic hepatitis-B related CLD especially in older men who are lean and have a higher viral load. These patients have lower BMD at spine and thereby are more prone for vertebral fractures which may add to their existing morbidity. Addition of Tenofovir worsens the femoral neck BMD and probably may increase the risk of more lethal, hip fractures. However, long-term follow-up studies are needed to study the efficacy of medical intervention in improving bone health in these individuals.

CONFLICTS OF INTEREST

The authors have none to declare.

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REFERENCES

1. AGA Clinical Practice Committee. AGA technical review on osteoporosis in hepatic disorders. *Gastroenterology.* 2003;125:941–966.
2. Collier JD, Ninkovic M, Compston JE. Guidelines on the management of osteoporosis associated with chronic liver disease. *Gut.* 2002;50(suppl 1):i1–i9.
3. Moschen AR, Kaser A, Stadlmann S, et al. The RANKL/OPG system and bone mineral density in patients with chronic liver disease. *J Hepatol.* 2005;43:973–983.
4. Gallejo-Rojo FJ, Gonzalez-Calvin JL, Munoz-Torres M, Mundi JL, Fernandez-Perez R, Rodrigo-Moneno D. Bone mineral density, serum insulin like growth factor I and bone turnover markers in viral cirrhosis. *Hepatology.* 1998;28:695–699.
5. Cemborain A, Castilla-Cortazar I, Garcia M, et al. Osteopaenia in rats with liver cirrhosis: beneficial effects of IGF-1 treatment. *J Hepatol.* 1998;28:122–131.
6. Cockayne S, Adamson J, Lanham-New S, Shearer MJ, Gilbody S, Torgerson DJ. Vitamin K and the prevention of fractures. *Arch Intern Med.* 2006;166:1256–1261.
7. Grigsby IF, Pham L, Mansky LM, Gopalakrishnan R, Mansky KC. Tenofovir-associated bone density loss. *Therap Clin Risk Manage.* 2010;6(1):41–47.
8. Diamond TH, Stiel D, Lunzer M, McDowall D, Eckstein RP, Posen S. Hepatic osteodystrophy. Static and dynamic bone histomorphometry and serum bone Gla-protein in 80 patients with chronic liver disease. *Gastroenterology.* 1989;96:213–221.
9. Kapoor N, Shetty S, Thomas N, Paul TV. Wilson's disease: an endocrine revelation. *Indian J Endocrinol Metab.* 2014;18(6):855–857. http://dx.doi.org/10.4103/2230-8210.141383.
10. Uretmen S, Gol M, Cimrin D, Irmak E. Effects of chronic liver disease on bone mineral density and bone metabolism markers in postmenopausal women. *Eur J Obstet Gynecol Reprod Biol.* 2005;123(November (1)):67–71.
11. Perrot S, Aslangul E, Szwebel T, Caillat-Vigneron N, Le Jeunne C. Bone pain due to fractures revealing osteomalacia related to tenofovir-induced proximal renal tubular dysfunction in a human immunodeficiency virus-infected patient. *J Clin Rheumatol.* 2009;15(2):72–74.
12. Lebrecht D, Venhoff AC, Kirschner J, Wiech T, Venhoff N, Walker UA. Mitochondrial tubulopathy in tenofovir disoproxil fumarate-treated rats. *J Acquir Immune Defic Syndr.* 2009;51(3):258–263.
13. Bedimo R, Cutrell J, Zhang S, et al. Mechanisms of bone disease in HIV and hepatitis C virus: impact of bone turnover, tenofovir exposure, sex steroids and severity of liver disease. *AIDS.* 2015 [Epub ahead of print].
14. Lucaci C, Lencu M, Acalovschi M. Risk factors for low mineral bone density in patients with chronic viral C infection. *J Hepatol.* 2012;56:S352.
15. Orsini LG, Pinheiro MM, Castro CH, Silva AE, Szejnfeld VL. Bone mineral density measurements, bone markers and serum vitamin D concentrations in men with chronic non-cirrhotic untreated hepatitis C. *PLOS ONE.* 2013;8(November (11)):e81652.
16. Lin JC, Hsieh TY, Wu CC, et al. Association between chronic hepatitis C virus infection and bone mineral density. *Calcif Tissue Int.* 2012;91(December (6)):423–429.
17. Saeedi R, Jiang SY, Holmes DT, Kendler DL. Fibroblast growth factor 23 is elevated in tenofovir-related hypophosphatemia. *Calcif Tissue Int.* 2014;94(June (6)):665–668.
18. Earle KE, Seneviratne T, Shaker J, Shoback D. Fanconi's syndrome in HIV^+ adults: report of three cases and literature review. *J Bone Miner Res.* 2004;19(May (5)):714–721.
19. Havens PL, Kiser JJ, Stephensen CB, et al. Adolescent Medicine Trials Network for HIV/AIDS Interventions (ATN) 063 Study Team. Association of higher plasma vitamin D binding protein and lower free calcitriol levels with tenofovir disoproxil fumarate use and plasma and intracellular tenofovir pharmacokinetics: cause of a functional vitamin D deficiency? *Antimicrob Agents Chemother.* 2013;57(November (11)):5619–5628.
20. Parés A, Guañabens N, Rodés J. Gene polymorphisms as predictors of decreased bone mineral density and osteoporosis in primary biliary cirrhosis. *Eur J Gastroenterol Hepatol.* 2005;17:311–315.
21. Boyle WJ, Scott-Simonet W, Lacey DL. Osteoclast differentiation and activation. *Nature.* 2003;423:337–342.
22. Nishiguchi S, Shimoi S, Kurooka H, et al. Randomized pilot trial of vitamin K2 for bone loss in patients with primary biliary cirrhosis. *J Hepatol.* 2001;35:543–545.
23. Goral V, Simsek M, Mete N. Hepatic osteodystrophy and liver cirrhosis. *World J Gastroenterol.* 2010;16:1639–1643.
24. Bell H, Raknerud N, Falch JA, Haug E. Inappropriately low levels of gonadotrophins in amenorrhoeic women with alcoholic and non-alcoholic cirrhosis. *Eur J Endocrinol.* 1995;132:444–449.
25. Hay JE, Guichelaar MM. Evaluation and management of osteoporosis in liver disease. *Clin Liver Dis.* 2005;9:747–766.

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