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Vitamin D deficiency and its relation to bone mineral density and liver fibrosis in HIV–HCV coinfection

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Abstract

Background—Fractures and cirrhosis are major causes of morbidity and mortality among HIV–HCV-coinfected individuals. It is not known whether vitamin D deficiency is associated with these outcomes.

Methods—Between 2005 and 2007, 116 HIV–HCV-coinfected individuals underwent dual-energy X-ray absorptiometry within 1 year of a liver biopsy. 25-Hydroxy-vitamin D (25OHD) and parathyroid hormone were measured from archived samples. Low bone mineral density (BMD) was defined as BMD 2 standard deviations lower than age-, sex- and race-matched controls (Z-score –2.0) at the total hip, femoral neck or lumbar spine. Histological fibrosis staging was assessed according to the METAVIR system (0 [no fibrosis] to 4 [cirrhosis]).

Results—The cohort was 87% African-American and 63% male. The median age (IQR) was 49.9 years (46.5–53.3). A total of 89% had a CD4⁺ T-cell count >200 cells/mm³ and 64% were receiving HAART. The median 25OHD was 19 ng/ml (IQR 11.0–26.0). Hypovitaminosis D (25OHD 15 ng/ml) was present in 41% and secondary hyperparathyroidism, defined by parathyroid hormone >65 pg/ml, was present in 24%. In total, 27% had low BMD (Z-score –2) at the spine, femoral neck or total hip, and 39% had significant hepatic fibrosis (METAVIR 2). In multivariate analysis, vitamin D deficiency was not associated with significant fibrosis or with BMD at any site.

Conclusions—Vitamin D deficiency was highly prevalent in this mostly African-American HIV–HCV-coinfected population, but was not related to BMD or liver disease severity. These data suggest that efforts to increase vitamin D levels in this population may not improve bone or liver outcomes.

Introduction

Vitamin D deficiency is a reversible risk factor that has been linked to severe fibrosis in HCV-infected patients [1,2] and may contribute to the high risk of fragility fractures among

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Disclosure statement

TTB has served as a consultant to Gilead, ViiV Healthcare and EMD-Serono, has received lecture fees from BMS, ViiV Healthcare and Tibotec, has developed educational presentations for ViiV Healthcare, Gilead and Tibotec and has received research support from Merck and GSK. All other authors declare no competing interests.

patients with HIV–HCV coinfection [3–5]. Lower vitamin D levels have been found to be independently associated with the severity of hepatic inflammation in patients with non-alcoholic fatty liver disease [2,6]. The objective of this study was to determine the prevalence and correlates of vitamin D deficiency in a cohort of HIV–HCV-coinfected individuals and to investigate whether vitamin D deficiency is associated with hepatic fibrosis or reduced bone mineral density (BMD).

Methods

Study population

Between January 2007 and February 2009, 179 individuals enrolled in a cohort, who had been recruited from the Johns Hopkins University HIV/HCV Clinic, (Baltimore, MD, USA) underwent BMD measurements of the hip and spine by dual-energy X-ray absorptiometry (DXA), as previously described [7]. Individuals were recruited into a prospective cohort whose primary aim was to characterize liver disease progression among HIV–HCV-coinfected individuals. For those without cirrhosis ($n=143$), the DXA was performed within 1 year of the liver biopsy. The median time between the liver biopsy and the DXA was 13 days (IQR 0–67 days). There were 13 known cirrhotics who underwent DXA scans and testing between January 2007 and May 2008, but not liver biopsy. Blood samples for measurement of total 25-hydroxyvitamin D (25OHD) and parathyroid hormone (PTH) were available for 116 individuals.

For all individuals, information on patient demographics, social practices, prescribed medications and laboratory parameters was obtained from clinical and laboratory databases [7]. The designation of injection drug use and alcohol abuse was based on physician diagnosis, whereas smoking was self-reported via an orally administered questionnaire. HAART was defined as use of a protease inhibitor, non-nucleoside reverse transcriptase inhibitor, fusion inhibitor or integrase inhibitor. The study was approved by the Johns Hopkins Institutional Review Board and written informed consent was obtained from all participants.

Laboratory evaluations

Patients had standard laboratory assessments, including serum chemistry panels, alanine transaminase levels, a CD4⁺ T-cell cell count and plasma HIV RNA level measured within 6 months of DXA. HCV genotype testing was performed using reverse transcriptase-PCR. Intact PTH was measured by immunochemiluminometric assay (Siemens IMMULITE® 2000 System Analyzer; Slanders, NJ, USA) by Quest Diagnostics (Baltimore, MD, USA) with interassay coefficient of variation of 6.3–9.1%. Vitamin D (25OHD) was measured by liquid chromatography, tandem mass spectrometry (LC/MS/MS) by Quest Diagnostics (interassay coefficient of variation: 7%). Hyperparathyroidism (HPT) was defined as a PTH greater than the upper limit of the normal range (>65 pg/ml), and vitamin D deficiency was defined as 25OHD < 15 ng/ml [8].

Liver histology

A transcutaneous liver biopsy was performed using an 18-gauge needle. After preparation, slides were evaluated by a single pathologist (MST), as previously described [7]. For the fibrosis stage, biopsies were evaluated according to the METAVIR system (0 [no fibrosis] to 4 [cirrhosis]) [9]. Significant fibrosis was defined as METAVIR grades 2, 3 or 4 [10].

Body composition and BMD

BMD at the total hip, femoral neck and lumbar spine were measured using a Hologic 4500A machine with QDA4500A software version 9.03 (Hologic Inc., Waltham, MA, USA). Z-

scores were calculated from the site-specific BMD measures using normative data from the manufacturer matched for gender and race [11,12]. The Z-score is the number of standard deviations a participant's BMD falls from the mean BMD of a gender-, age- and race-matched population. Low BMD was defined as Z-score ≤ -2 at the spine, total hip or femoral neck in accordance with National Osteoporosis Foundation guidelines [13].

Statistical analyses

Descriptive analysis was used to define the characteristics of the population and to determine the prevalence of vitamin D deficiency, HPT, low BMD and fibrosis. In the first analysis, correlates of vitamin D deficiency (< 15 ng/ml) were explored. As the prevalence of vitamin D deficiency was high, Poisson regression with robust variance estimation was used to assess the correlates of vitamin D deficiency. Correlates of interest included age, sex, race, body mass index, season, history of alcohol abuse or injection drug use, smoking status, CD4⁺ T-cell count, HIV RNA level and use of HAART. As certain classes of antiretroviral therapy have been associated with BMD reduction [14], the effects of different drug classes (protease inhibitors, non-nucleoside reverse transcriptase inhibitors and nucleoside reverse transcriptase inhibitors at the time of DXA or before) as well as specific drugs (zidovudine, stavudine, tenofovir disoproxil fumarate, efavirenz at the time of DXA or before) were explored. The effect of prior HCV treatment on vitamin D deficiency (no individuals were on HCV treatment at the time of DXA) was also explored. Covariates associated with vitamin D deficiency at $P < 0.10$ were considered for inclusion in the multivariable model. In addition, linear regression was used to assess the correlation between vitamin D and PTH. In the second analysis, the association between vitamin D deficiency (< 15 ng/ml), significant liver fibrosis (METAVIR ≥ 2) and bone (site-specific Z-scores) outcomes was explored. As the prevalence of liver fibrosis was high, Poisson regression with robust variance estimation was used to determine the independent association between vitamin D deficiency and significant fibrosis. Linear regression models were used to determine the independent associations between vitamin D deficiency and each of the three site-specific (spine, hip and femoral neck) Z-scores. All models were adjusted for age, sex, race, body mass index, smoking, history of alcohol abuse, history of injection drug use, HIV RNA, CD4⁺ T-cell count, HAART use (ever/never) and HPT. In a sensitivity analysis, individuals with impaired or unknown renal function (glomerular filtration rate [GFR] < 60 ml/min/1.73 m²) were excluded, but this did not significantly alter any of the results.

Results

Population demographics

The cohort ($n=116$) was predominantly African- American (87%) and consisted of 63% males, with a median age of 49.9 years (IQR 46.5–53.3). Data on social habits, biochemical measures and HAART are presented in Table 1. A total of 91% of patients (104/114; 2 missing) had GFR > 60 ml/min/1.73 m².

Correlates of vitamin D deficiency

There was a modest inverse relation between 25OHD and PTH level ($r^2=0.046$, $P=0.02$). In multivariate analysis, vitamin D deficiency was significantly more common in winter (prevalence ratio [PR] 1.97; 95% CI 1.06, 3.66) and spring (PR 2.02; 95% CI 1.14, 3.57) as compared with summer, whereas being overweight (PR 0.40; 95% CI 0.20, 0.79), White (PR 0.28; 95% CI 0.10, 0.79), older (PR 0.83 per 5 year increment; 95% CI 0.69, 0.99) and having a history of injection drug use (PR 0.64; 95% CI 0.42, 0.98) were protective.

Vitamin D and low BMD

The prevalence of low BMD (30% among those with versus 25% among those without vitamin D deficiency; $P=0.57$) and mean Z-scores were similar among those with and without vitamin D deficiency (Figure 1A). Similarly, there was no significant difference in the prevalence of low BMD among those with and without HPT (32% versus 25%; $P=0.46$). Vitamin D deficiency was not independently associated with lower Z-scores at any site after adjustment for other confounders. Adjusted estimates (SE) for vitamin D deficiency at site-specific Z-scores were as follows: total hip 0.19 (0.22; $P=0.39$) spine 0.52 (0.35; $P=0.14$) and femoral neck 0.05 (0.22; $P=0.82$). Similar results were obtained when alternate cut points (≥ 10 ng/ml and ≥ 20 ng/ml) to define vitamin D deficiency were used.

Vitamin D and fibrosis

The prevalence of significant fibrosis (grade ≥ 2) was similar among those with and without vitamin D deficiency (43% versus 35%; $P=0.43$; Figure 1B). Similarly, the prevalence of fibrosis was similar in those with and without HPT (32% versus 41%; $P=0.41$). Vitamin D deficiency was not independently associated with significant fibrosis after adjusting for other confounders (adjusted PR [95% CI] 1.37 [0.77, 2.44]).

Discussion

Vitamin D deficiency has been described in HCV-infected patients [1,2] and HIV-infected individuals [15], but it is unknown whether vitamin D deficiency in these high-risk populations results in adverse health consequences. In this middle-aged, predominately African-American cohort of HIV–HCV-coinfected individuals, we found a high prevalence of vitamin D deficiency (41%), hepatic fibrosis (39%) and low BMD (27%); however, vitamin D deficiency was not related to hepatic fibrosis or low BMD.

Vitamin D plays an important role in bone metabolism and vitamin D deficiency has been associated with lower BMD in Caucasian populations [16,17]. In African-American men and women, however, lower vitamin D levels have not been associated with low BMD [18], despite the fact that average vitamin D levels are lower than Caucasians, because of decreased vitamin D synthesis [19], reduced vitamin D intake [20] and other socioeconomic and genetic factors [21]. Racial differences in the effect of vitamin D on bone health have been highlighted in a recent case-control study from the Women's Health Initiative, which showed a detrimental effect of lower 25OHD levels on fractures in Caucasian women, but a protective effect (that is lower 25OHD levels [≥ 20 ng/ml] associated with a lower risk of fracture) in African-American women [22]. A potential mechanism explaining this phenomenon is that African-Americans could be more resistant to the bone-resorbing effects of PTH [23].

We also found no relation between vitamin D deficiency and hepatic fibrosis. Vitamin D deficiency could play a role in inflammation and fibrosis through release of inflammatory and profibrogenic cytokines [6] and indeed has been linked to severe fibrosis in HCV-infected patients [1,2]. In addition, vitamin D deficiency has also been postulated to cause an unfavourable response to HCV antiviral therapy [1,24]. Further studies to assess the role of vitamin D in liver disease should examine the longitudinal relation between vitamin D levels, supplementation and progression of fibrosis, particularly with respect to differential effects by race.

Our study has a few limitations. Because it was cross-sectional, we cannot conclude that vitamin D deficiency would not potentially have an adverse effect on BMD or fibrosis in this cohort of HIV–HCV-coinfected individuals. We did not have fracture history on these individuals; however, follow-up for future fracture events could add information on the

clinical significance of low BMD in this population. In addition, our sample size was relatively small, and the study could potentially have been underpowered to detect small differences in BMD and fibrosis by vitamin D deficiency status. With a sample size of 116, prevalence of vitamin D deficiency of 41% and standard deviation in Z-score of 1.0 (at all three sites), we would have 80% power to detect a difference in mean Z-score between those with and without vitamin D deficiency of 0.34. However, even at the hip where the observed difference in mean Z-score between those with and without vitamin D deficiency was the largest (estimated mean difference -0.06 ; SE 0.20), if the observed effects of vitamin D were real, it would not be clinically significant.

In conclusion, vitamin D deficiency was prevalent among predominately African-American, HIV-HCV-coinfected individuals but was not related to BMD or fibrosis. It is questionable whether vitamin D supplementation in such populations would provide any benefits, and whether African-Americans have vitamin D thresholds different from Caucasians. Further studies examining the effect of vitamin D supplementations in African-Americans with multiple comorbidities should assess potential benefits or harms of vitamin D replacement.

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Figure 1.

The relationship between bone mineral density Z-score and hepatic fibrosis to 25OHD in HIV–HCV-coinfected patients.

(A) Mean Z-scores ($\pm$ SD) by vitamin D (25-hydroxyvitamin D [25OHD]) deficiency status (cutoff 15 ng/ml). (B) Distribution of 25OHD by fibrosis stage: no fibrosis (Metavir 0,1) versus fibrosis (Metavir 2,3,4).

Table 1Demographic and clinical characteristics of the study population and correlates of vitamin D deficiency^a

Variable	Value	Crude prevalence ratio for vitamin D deficiency (95% CI) ^b	Adjusted prevalence ratio for vitamin D deficiency (95% CI) ^b
Female	43 (37)	1.35 (0.88, 2.09)	1.12 (0.76, 1.64)
Median age, years (IQR)	49.9 (46.5–53.3)	–	–
Age, per 5 years	–	0.85 (0.71, 1.03)	0.83 (0.69, 0.99)
White	15 (13)	0.30 (0.08, 1.10)	0.28 (0.10, 0.79)
History of alcohol abuse	53 (46)	1.33 (0.86, 2.06)	–
History of injection drug use	85 (73)	0.65 (0.42, 1.00)	0.64 (0.42, 0.98)
Currently smoking	70 (63)	1.14 (0.71, 1.85)	–
Season			
Winter (Dec, Jan, Feb)	22 (19)	2.00 (1.01, 3.93)	1.97 (1.06, 3.66)
Spring (Mar, Apr, May)	39 (34)	2.03 (1.08, 3.79)	2.02 (1.14, 3.57)
Summer (Jun, Jul, Aug)	33 (28)	1	1
Autumn (Sep, Oct, Nov)	22 (19)	0.83 (0.32, 2.16)	0.77 (0.32, 1.82)
Body mass index			
Normal, BMI <25 kg/m ²	59 (52)	1	1
Overweight, BMI 25–29 kg/m ²	30 (26)	0.41 (0.19, 0.89)	0.40 (0.20, 0.79)
Obese, BMI ≥30 kg/m ²	25 (22)	0.91 (0.54, 1.53)	0.72 (0.45, 1.14)
HIV RNA >400 copies/ml	23 (21)	1.46 (0.90, 2.37)	–
CD4 ⁺ T-cell count <200 cells/μl	12 (11)	1.39 (0.76, 2.51)	–
Never been on HAART ^c	11 (9)	1.38 (0.77, 2.50)	–
Currently not on HAART	42 (36)	1.22 (0.79, 1.90)	–
Currently on tenofovir	40 (34)	0.88 (0.54, 1.42)	–
Ever been on tenofovir	75 (65)	0.86 (0.55, 1.34)	–
Currently on efavirenz	23 (20)	0.82 (0.45, 1.51)	–
Ever been on efavirenz	66 (57)	0.74 (0.48, 1.14)	–
Currently on HCV therapy ^d	0 (0)	–	–
Ever been on HCV therapy	26 (22)	0.60 (0.31, 1.18)	–
Median total 25OHD, ng/ml (IQR) ^e	19 (11–26)	–	–
Hypovitaminosis D, 15 ng/ml	47 (41)	–	–
Median PTH, pg/ml (IQR)	43.5 (30–64.5)	–	–
Secondary hyperparathyroidism, PTH >65 pg/ml	28 (24)	–	–
Significant fibrosis, Metavir 2 ^f	45 (39)	1.20 (0.77, 1.85)	–
Low BMD at any site, Z-score <−2	31 (27)	–	–
Median Z-score at total hip (IQR)	−0.42 (−1.12–0.25)	–	–
Median Z-score at femoral neck (IQR)	−0.09 (−0.84–0.47)	–	–
Median Z-score at spine (IQR)	−0.95 (−1.83–0.16)	–	–

The total study population was $n=116$, and demographic and clinical characteristics were measured at the time of DEXA. All data are presented as n (%) unless indicated otherwise.

^aVitamin D deficiency is defined as 25-hydroxyvitamin D (25OHD) $\leq$ 15 ng/ml.

^bBold in crude column indicates $P < 0.1$; bold in adjusted column indicates $P < 0.05$.

^cHAART was defined as use of a protease inhibitor, non-nucleoside reverse transcriptase inhibitor, fusion inhibitor or integrase inhibitor.

^dDefined as combined interferon/ribavirin or ribavirin monotherapy.

^eMeasured as 25-hydroxyvitamin D2 and D3 (ng/ml).

^fMETAVIR system: 0 (no fibrosis) to 4 (cirrhosis). Significant fibrosis was defined as METAVIR grades 2, 3 or 4. BMD, bone mineral density; BMI, body mass index; PTH, parathyroid hormone.