

Hyperparathyroidism and Complications Associated with Vitamin D Deficiency in HIV-Infected Adults in New York City, New York

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Abstract

Although recent studies report a high prevalence of vitamin D deficiency in HIV-infected adults similar to that in the general population, metabolic complications of vitamin D deficiency may be worsened with HIV infection and remain insufficiently characterized. We conducted a retrospective cross-sectional cohort study to determine prevalence and correlates of vitamin D deficiency and hyperparathyroidism among HIV-infected patients attending an urban clinic. Vitamin D deficiency was defined as 25(OH)-vitamin D <20 ng/ml and insufficiency as 20 to <30 ng/ml, and hyperparathyroidism as parathyroid-hormone >65 pg/ml. We used the χ^2 test to compare proportions and logistic regression to assess for associations. Among 463 HIV-infected patients, the prevalence of vitamin D deficiency was 59%. The prevalence of hyperparathyroidism was 30% among patients with vitamin D deficiency, 23% among those with insufficiency, and 12% among those with sufficient vitamin D levels. Vitamin D deficiency was associated with increased odds of hyperparathyroidism. Severe vitamin D deficiency was associated with elevated alkaline phosphatase, a marker for increased bone turnover. Although efavirenz use was associated with vitamin D deficiency, and protease inhibitor use with decreased odds of vitamin D deficiency, there was no statistical difference in rates of hyperparathyroidism stratified by combination antiretroviral therapy (cART) use. Given the increased risk of osteopenia with HIV infection and cART use, vitamin D supplementation for all HIV-infected patients on cART should be prescribed in accordance with the 2011 Endocrine Society guidelines. In HIV-infected patients with severe vitamin D deficiency or hyperparathyroidism, screening for osteomalacia and osteopenia may be warranted.

Introduction

HIV INFECTION AND COMBINATION antiretroviral therapy (cART) increase the risk of metabolic complications such as bone disorders (i.e., osteopenia, osteoporosis, and avascular necrosis), dyslipidemia, and glucose intolerance.^{1–5} Use of cART increased the odds of osteopenia by 2.5-fold,⁶ and initiation of cART decreased bone mineral density (BMD) by 2–6% within the first 2 years.^{4,7} A meta-analysis of cross-sectional cohorts found that HIV infection increased the odds of osteopenia by 6-fold and of osteoporosis by nearly 4-fold.⁶ In addition to osteopenia and osteoporosis, HIV infection was associated with higher rates of fractures, including vertebral, hip, and wrist fractures in several studies,^{8–11} though not among premenopausal women.¹²

Recent studies report a high prevalence of vitamin D deficiency in HIV-infected adults.^{13–15,17} Analysis of data from the Study to Understand the Natural History of HIV and AIDS in

the Era of Effective Therapy (SUN), a large, prospective observational cohort study, found 70% prevalence of vitamin D insufficiency or deficiency among 672 HIV-infected adults.¹⁶ Although adults surveyed in the U.S. National Health and Nutrition Examination Survey (NHANES) had a similar prevalence of vitamin D deficiency,¹⁶ in HIV-infected adults the metabolic complications of vitamin D deficiency may be worsened by additive or synergistic effects with HIV infection and cART use.

The range of complications associated with vitamin D deficiency overlaps with known metabolic complications of HIV infection and cART use. Vitamin D deficiency results in secondary hyperparathyroidism, osteomalacia, osteoporosis, muscle weakness, and increased fracture risk in adults.^{18,19} In addition, vitamin D deficiency may also play a role in insulin resistance, the metabolic syndrome, and immunomodulation.^{18,20} Furthermore, metabolic complications (e.g., decreased BMD) associated with use of cART may be mediated in part by alterations in vitamin D metabolism through the

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effect of cART on the cytochrome P450 system.²¹ The use of efavirenz (EFV) in HIV-infected adults has been demonstrated to decrease 25(OH)-vitamin D levels and increase the risk of vitamin D deficiency,^{16,22–25} while the use of protease inhibitors (PIs), in contrast, was associated with increased 25(OH)-vitamin D levels.^{6,16} The spectrum and severity of metabolic complications associated with vitamin D deficiency in HIV-infected adults remain to be better characterized.

We conducted a retrospective cross-sectional cohort study to determine prevalence, associated risk factors, and complications associated with vitamin D deficiency in an HIV clinic population in New York City. We compared proportions of patients with hyperparathyroidism stratified by level of vitamin D deficiency, and evaluated current use of cART as a risk factor for vitamin D deficiency and hyperparathyroidism.

Materials and Methods

The study included HIV-infected persons age ≥ 18 years who received routine HIV care at Bellevue Virology Clinic from October 2009 through March 2010, and who had serum 25(OH)-vitamin D measured as part of a clinic-wide quality-improvement project initiated to assess for prevalence of vitamin D deficiency and to determine whether a therapeutic intervention was warranted for the clinic population. Patients with \geq stage 3 chronic kidney disease (CKD, defined as estimated GFR < 60 ml/min/1.73m²) were excluded, as CKD is known to interfere with vitamin D metabolism. We extracted data on patient demographics, current medications including nutritional supplements, duration of antiretroviral use, HIV viral load, CD4 cell count, serum 25(OH)-vitamin D, parathyroid hormone (PTH), and other concurrent laboratory results. Study approval was received from the NYU School of Medicine, Bellevue Hospital Center and the central office of the New York City Health and Hospital Corporation.

Plasma 25(OH)-vitamin D was measured using liquid chromatography, tandem mass spectrometry (Quest Diagnostics, Madison, NJ). PTH was measured using solid-phase chemiluminescent immunoassay (Immulinite 2000, Siemens Healthcare Diagnostics, Deerfield, IL). HIV viral load was measured using the Amplicor HIV-1 Monitor v1.5 Ultra-sensitive Assay (Roche Diagnostics, Branchburg, NJ).

The primary endpoint was vitamin D deficiency, defined as 25(OH)-vitamin D < 20 ng/ml.¹⁸ Secondary endpoints included vitamin D insufficiency, defined as 25(OH)-vitamin D 20 to < 30 ng/ml, and vitamin D sufficiency, defined as 25(OH)-vitamin D ≥ 30 ng/ml.¹⁸ Severe vitamin D deficiency was defined as 25(OH)-vitamin D < 12 ng/ml, a level of deficiency associated with increased risk for adverse effects on bone health.²⁶

Hyperparathyroidism was defined as PTH greater than 65 pg/ml, elevated alkaline phosphatase as serum alkaline phosphatase greater than 100 U/liter, and hypocalcemia as serum albumin-adjusted calcium level of less than 9.0 mg/dl. In accordance with American Diabetes Association 2011 guidelines, prediabetes was defined as having glycated hemoglobin A(1c) (HbA1c) 5.7–6.4%, and diabetes as having HbA1c $\geq 6.5\%$ or being on antihyperglycemics.²⁷ HIV viral load cut-offs were defined at 200 copies/ml, in accordance to the 2011 DHHS HIV Guidelines, and at 50 copies/ml, the lower limit of detection of the assay.²⁸

We calculated the prevalence of vitamin D deficiency, severe deficiency, insufficiency, and sufficiency in our study population. Data were analyzed using SPSS 19 statistical software. Comparisons of proportions were made using chi-square testing. Student's *t*-test, ANOVA with Bonferroni and Tukey's post-hoc tests, and linear regression were used to assess for correlates of 25(OH)-vitamin D level, and univariate logistic regression to assess for factors associated with vitamin D deficiency. Variables with a *p*-value ≤ 0.10 in univariate analysis were included in multivariate logistic regression, and adjusted odds ratios (aORs) and corresponding 95% confidence intervals (CIs) were calculated.

Results

Baseline characteristics

Of 507 patients, 44 were excluded due to evidence of stage ≥ 3 CKD, and 463 were included in the analysis. Seventy-six percent ($N = 351$) were male, 48% ($N = 221$) were black, and the median age was 46 years [interquartile range (IQR) 38–53 years] (Table 1). Eighty-two percent of patients were currently on cART ($N = 381$). Among patients not on cART, 52% (43/82) were cART-naive and 48% (39/82) were self-reported non-adherent to cART in the medical record. Sixty-four percent of patients had a CD4 T cell count > 350 cells/mm³ ($N = 296$), and 72% ($N = 333$) had a viral load ≤ 200 copies/ml, including 65% ($N = 300$) with a viral load < 50 copies/ml (Table 2). Mean and median CD4 T cell counts were 455 cells/mm³ and 433 cells/mm³, respectively (IQR 365–617 cells/mm³).

Thirty percent ($N = 140$) of patients were on an EFV-based regimen, of which 88% (123/140) had a viral load ≤ 200 copies/ml and 79% (110/140) had been on EFV ≥ 12 months. Forty-three percent ($N = 201$) were on a protease inhibitor (PI)-based regimen, of which 97% (195/201) were on ritonavir-boosted PI (rPI), 84% (168/201) had a viral load ≤ 200 copies/ml, and 70% (141/201) had been on an rPI-based regimen ≥ 12 months (Table 1). Thirty-four percent ($N = 156$) of patients were taking multivitamins (MVI), and 4% ($N = 17$) vitamin D supplementation. Among patients on MVI, 12% (18/156) had detectable vitamin D₂, the form of vitamin D associated with supplementation, compared to 5% (14/306) in patients not on MVI ($p = 0.005$). Among patients on vitamin D supplementation, 29% (5/17) had detectable vitamin D₂, compared to 7% (30/445) among those not on vitamin D supplementation ($p = 0.005$). Use of MVI or vitamin D supplementation was associated with age ≥ 35 years ($p < 0.001$), use of EFV-based cART ($p = 0.005$), and use of PI-based cART ($p = 0.009$). The use of other medications known to interfere with vitamin D metabolism, such as anticonvulsants (4%, $N = 17$), antimycobacterial drugs (2%, $N = 8$; i.e., isoniazid, ethambutol, pyrazinamide, or rifamycins), and corticosteroids (0.4%, $N = 2$), was minimal. Eighty-seven percent ($N = 401$) of patients had vitamin D levels measured during the winter season, from December through February. Nine percent ($N = 40$) of patients also had chronic hepatitis B infection (i.e., hepatitis B surface antigen or RNA positivity), and 22% ($N = 102$) were coinfecting with hepatitis C (i.e., hepatitis C antibody positivity).

25(OH)-vitamin D levels and vitamin D deficiency

Median 25(OH)-vitamin D level was 17 ng/ml (IQR 10–25 ng/ml). Fifty-nine percent ($N = 275$) of patients were

TABLE 1. BASELINE CHARACTERISTICS BY LEVEL OF VITAMIN D DEFICIENCY

Variable	All N=463	Vitamin D sufficient N=77	Vitamin D insufficient N=111	Vitamin D deficient N=275
Age, years**				
Median (IQR)	46 (38–53)	48 (43–56)	47 (40–52)	44 (36–53)
Age in years by strata, no. (%)†				
18–34	84 (18)	4 (5)	13 (12)	64 (24)
35–44	125 (27)	21 (27)	33 (30)	71 (26)
45–54	152 (33)	30 (39)	41 (37)	81 (30)
≥55	101 (22)	22 (29)	23 (21)	56 (20)
Sex, no. (%)				
Male	351 (76)	53 (69)	85 (77)	213 (78)
Race or ethnicity, no. (%)‡				
White	26 (6)	7 (9)	6 (5)	13 (5)
Black	221 (48)	26 (34)	42 (38)	153 (56)
Hispanic	160 (35)	34 (44)	44 (40)	82 (30)
Asian	40 (9)	6 (8)	13 (12)	21 (8)
Other	16 (4)	4 (5)	6 (5)	6 (2)
Season, no. (%)				
Fall	46 (10)	13 (17)	4 (4)	29 (11)
Winter	401 (87)	61 (79)	104 (94)	236 (86)
Spring	15 (3)	3 (4)	3 (3)	9 (3)
Weight, kg*				
Median (IQR)	74 (66–86)	72 (63–86)	73 (66–82)	75 (66–86)
Current medications, no. (%)				
Anticonvulsants	17 (4)	4 (5)	4 (4)	9 (3)
Antimycobacterial	8 (2)	1 (1)	2 (2)	5 (2)
MVI†	156 (34)	41 (53)	55 (50)	60 (22)
Vitamin D‡	17 (4)	8 (10)	7 (6)	2 (1)
Current antiretroviral therapy, no. (%)‡				
None	82 (17.7)	9 (11.7)	16 (14.4)	57 (20.7)
EFV-based >1 year	110 (23.8)	15 (19.5)	26 (23.4)	69 (25.1)
EFV-based <1 year	30 (6.5)	3 (3.9)	6 (5.4)	21 (7.6)
PI-based >1 year	145 (31.3)	36 (46.8)	38 (34.2)	71 (25.8)
PI-based <1 year	56 (12.1)	9 (11.7)	10 (9.0)	37 (13.5)
Other	40 (8.6)	5 (6.5)	15 (13.5)	20 (7.3)
Current NRTI ≥12 months, no. (%)				
Abacavir	42 (9.1)	10 (13)	10 (9.0)	22 (8.0)
Emtricitabine	203 (44)	37 (48)	51 (46)	115 (42)
Lamivudine	71 (15)	13 (17)	22 (20)	36 (13)
Tenofovir	235 (51)	44 (57)	58 (52)	133 (48)

* $p < 0.10$ and ** $p < 0.05$ by *t*-test, linear regression, or ANOVA as appropriate for 25(OH)-vitamin D level.

† $p < 0.05$ by Chi-square test comparing proportion with and without vitamin D deficiency.

IQR, interquartile range; MVI, multivitamin; EFV, efavirenz; PI, protease inhibitor; NRTI, nucleoside reverse transcription inhibitor.

vitamin D deficient, 24% ($N=111$) vitamin D insufficient, and 17% ($N=77$) vitamin D sufficient (Table 1). Among patients with vitamin D deficiency, 49% (135/275) had severe vitamin D deficiency [i.e., 25(OH)-vitamin D < 12 ng/ml].

Factors associated with increased odds of vitamin D deficiency included younger age ($p=0.001$) and black race/ethnicity [odds ratio (OR) 2.18, 95% confidence interval (CI): 1.49–3.19] (Tables 1 and 3). Hyperparathyroidism (OR 1.86, 95% CI: 1.16–3.01) and having prediabetes or diabetes (OR 1.514, 95% CI: 1.01–2.27) were associated with increased odds of vitamin D deficiency (Tables 2 and 3). Additionally, current CD4 T cell count < 200 cells/mm³ (OR 2.26, 95% CI: 1.26–3.94) and current viral load > 200 copies/ml (OR 2.79, 95% CI: 1.76–4.41) were associated with increased odds of vitamin D deficiency (Tables 2 and

3); serum 25(OH)-vitamin D levels directly correlated with CD4 T cell count ($p=0.002$) and inversely correlated with HIV viral load ($p=0.001$).

Current use of MVI (OR 0.27, 95% CI: 0.18–0.40), vitamin D supplements (OR 0.08, 95% CI: 0.02–0.37), and PI-based cART regimen (OR 0.51, 95% CI: 0.30–0.88) was associated with decreased odds of vitamin D deficiency (Tables 1 and 3). There was insufficient sample size to evaluate the effects of any individual PI. Current use of EFV-based cART was not significantly associated with vitamin D deficiency (OR 0.79, 95% CI: 0.44–1.42) in univariate analysis. Analysis by type of nucleoside reverse transcriptase inhibitor used for ≥12 months did not find an association of vitamin D deficiency with use of abacavir ($N=42$, $p=0.324$), emtricitabine ($N=203$, $p=0.288$), lamivudine ($N=71$, $p=0.107$), or tenofovir ($N=235$, $p=0.213$).

TABLE 2. METABOLIC COMPLICATIONS, VIRAL LOAD, AND IMMUNOLOGIC STATUS BY LEVEL OF VITAMIN D DEFICIENCY

Variable	All N=463	Vitamin D sufficient N=77	Vitamin D insufficient N=111	Vitamin D deficient N=275
Parathyroid hormone level, pg/ml**				
Median (IQR)	46 (31–65)	40 (26–57)	45 (29–61)	49 (34–73)
Hyperparathyroidism,† no. (%)				
PTH > 65 pg/ml	104 (26)	8 (12)	23 (23)	73 (30)
PTH ≤ 65 pg/ml	301 (74)	57 (88)	76 (77)	168 (70)
Elevated alkaline phosphatase, hypocalcemia, no. (%)				
Alk phos > 100 U/liter	165 (36)	27 (35)	32 (29)	106 (39)
Adjusted calcium < 9.0 mg/dl	188 (41)	30 (39)	43 (39)	115 (42)
Prediabetes or diabetes, no. (%)‡				
HbA1c ≥ 5.7%‡	162 (35)	18 (25)	38 (37)	106 (41)
Current viral load, no. (%)‡				
< 50 copies/ml	300 (65)	59 (77)	84 (76)	157 (57)
50–200 copies/ml	33 (7)	4 (5)	9 (8)	20 (7)
> 200 copies/ml	130 (28)	14 (18)	18 (16)	98 (36)
Current CD4 T cell count, no. (%)‡				
> 350 cells/mm ³	296 (64)	52 (68)	78 (70)	166 (60)
200–350 cells/mm ³	94 (20)	16 (21)	23 (21)	55 (20)
< 200 cells/mm ³	73 (16)	9 (12)	10 (9.0)	54 (20)

** $p < 0.05$ by t -test, linear regression, or ANOVA as appropriate for 25(OH)-vitamin D level.

† $p < 0.05$ by Chi-square test comparing proportion with and without vitamin D deficiency.

IQR, interquartile range; PTH, parathyroid hormone; Alk phos, alkaline phosphatase.

Vitamin D deficiency was not associated with chronic hepatitis B ($p = 0.958$) or hepatitis C infection ($p = 0.773$).

Multivariate analysis

We included age, black race, weight by quartiles, current use of MVI, current use of vitamin D supplements, CD4 count, viral load, and current antiretroviral therapy in our multivariate logistic regression model (Table 3). Black race [adjusted OR (aOR) 2.26, 95% CI: 1.45–3.54] was independently associated with significantly higher odds of vitamin D deficiency. Age 35–54 years ($p < 0.05$), current use of MVI (aOR 0.27, 95% CI: 0.17–0.42), and current use of vitamin D supplements (aOR 0.13, 95% CI: 0.03–0.64) were independently associated with lower odds of vitamin D deficiency. Current CD4 cell count < 200 cells/mm³ was not significant ($p = 0.241$), but current viral load > 200 copies/ml (aOR 2.97, 95% CI: 1.48–5.97) remained independently associated with increased odds of vitamin D deficiency. Current use of EFV-based cART (aOR 3.06, 95% CI: 1.32–7.13) was an independent risk factor for vitamin D deficiency, while current use of PI-based cART ($p = 0.308$) was not significantly associated.

Hyperparathyroidism

Eighty-seven percent (405/463) of patients had serum PTH measured concurrently with serum 25(OH)-vitamin D. Of patients with documented serum PTH, the prevalence of hyperparathyroidism was 30% (73/241) among patients with vitamin D deficiency, 23% (23/99) among those with vitamin D insufficiency, and 12% (8/65) among those vitamin D sufficient (Table 2 and Fig. 1). Among patients with severe vitamin D deficiency, 38% (43/113) had hyperparathyroidism. Mean and median 25(OH)-vitamin D levels among patients

with hyperparathyroidism were 15.1 ng/ml and 13.0 ng/ml, respectively (IQR 8.0–21.0 ng/ml). Vitamin D deficiency doubled the odds of hyperparathyroidism (OR 1.86, 95% CI: 1.16, 3.01). After adjusting for vitamin D deficiency, current use of EFV-based ($p = 0.91$) or PI-based cART ($p = 0.12$) did not increase the risk of hyperparathyroidism when compared with no cART. Compared to current use of PI-based cART, current use of EFV-based cART had increased odds of hyperparathyroidism (OR 1.66, 95% CI: 0.993, 2.785; $p = 0.053$), though this difference was not statistically significant. After adjusting for vitamin D deficiency, there was no difference between use of PI-based or EFV-based cART in odds of hyperparathyroidism ($p = 0.111$). Current use of tenofovir ≥ 12 months ($p = 0.09$) was not associated with hyperparathyroidism.

We assessed alkaline phosphatase and albumin-adjusted calcium concentrations as indicators of increased bone turnover associated with vitamin D deficiency and hyperparathyroidism. Severe vitamin D deficiency (OR 1.63, 95% CI: 1.08, 2.45; $p = 0.02$), but not vitamin D deficiency ($p = 0.12$), was associated with elevated alkaline phosphatase. Hyperparathyroidism increased the odds of having elevated alkaline phosphatase (OR 1.57, 95% CI: 0.996, 2.475; $p = 0.052$), though this association did not reach statistical significance. Neither vitamin D deficiency ($p = 0.32$) nor severe vitamin D deficiency ($p = 0.21$) was associated with albumin-adjusted calcium concentration.

Glucose intolerance

Vitamin D deficiency was associated with being prediabetic or diabetic ($p = 0.044$, OR 1.51, 95% CI: 1.01, 2.27). In our cohort, being in the top quartile by weight (≥ 86 kg) was associated with being prediabetic or diabetic ($p = 0.002$). After adjusting for weight by quartile, there remained a trend toward increased

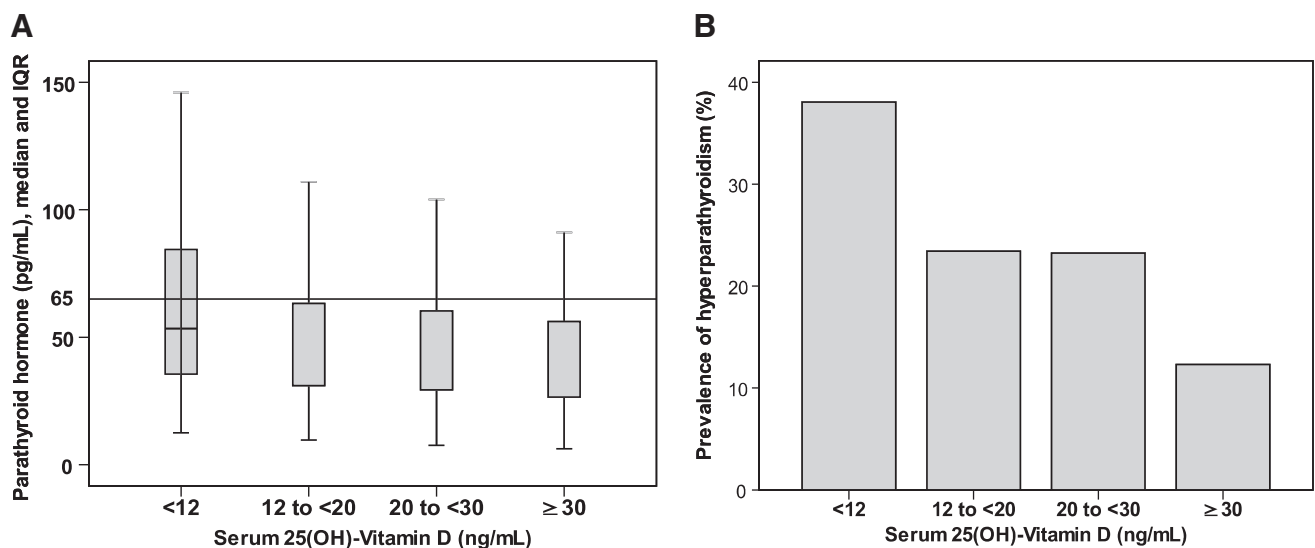
TABLE 3. CHARACTERISTICS ASSOCIATED WITH VITAMIN D DEFICIENCY

Variable	Odds ratio for vitamin D deficiency (95% CI)	p-value ^a	Adjusted odds ratio for vitamin D deficiency ^b (95% CI)	p-value ^c
Age, years				
18–34	Ref	Ref	Ref	Ref
35–44	0.33 (0.18, 0.63)	<0.01	0.41 (0.20, 0.83)	0.01
45–54	0.29 (0.16, 0.54)	<0.01	0.35 (0.18, 0.71)	<0.01
≥55	0.32 (0.16, 0.61)	<0.01	0.50 (0.24, 1.06)	0.07
Black race				
No	Ref	Ref	Ref	Ref
Yes	2.18 (1.49, 3.19)	<0.01	2.26 (1.45, 3.54)	<0.01
Weight				
1st quartile	Ref	Ref	Ref	Ref
2nd quartile	0.82 (0.49, 1.37)	0.44	0.80 (0.44, 1.46)	0.47
3rd quartile	1.23 (0.73, 2.08)	0.45	1.22 (0.66, 2.27)	0.52
4th quartile	1.19 (0.70, 2.00)	0.52	1.07 (0.58, 1.97)	0.84
Current medications				
MVI	0.27 (0.18, 0.40)	<0.01	0.27 (0.17, 0.42)	<0.01
Vitamin D	0.08 (0.02, 0.37)	<0.01	0.13 (0.03, 0.64)	0.01
Current CD4 T cell count, cells/mm³				
>350	Ref	Ref	Ref	Ref
200–350	1.10 (0.69, 1.77)	0.68	0.77 (0.45, 1.33)	0.35
<200	2.23 (1.26, 3.94)	0.01	1.50 (0.76, 2.93)	0.24
Current viral load, copies/mL				
<50	Ref	Ref	Ref	Ref
50–200	1.40 (0.67, 2.92)	0.37	1.13 (0.49, 2.60)	0.77
>200	2.79 (1.76, 4.41)	<0.01	2.97 (1.48, 5.97)	<0.01
Current antiretroviral therapy, no. (%)				
None	Ref	Ref	Ref	Ref
EFV-based	0.79 (0.44, 1.42)	0.43	3.06 (1.32, 7.13)	0.01
PI-based	0.51 (0.30, 0.88)	0.02	1.50 (0.69, 3.29)	0.31
Other	0.44 (0.20, 0.96)	0.04	1.82 (0.65, 5.07)	0.25

^aUnivariate analysis of association with vitamin D deficiency.

^bVariables associated with vitamin D deficiency with $p \leq 0.10$ were included in multivariate logistic regression (i.e., age, black race/ethnicity, current use of MVI, current use of vitamin D supplements, current CD4 count, current viral load, current antiretroviral therapy).

^cMultivariate analysis of association with vitamin D deficiency.



Top of box = 75th percentile. Bottom of box = 25th percentile. Outliers not shown.

FIG. 1. (A) Median serum parathyroid hormone (PTH) (pg/ml) by serum 25(OH)-vitamin D subgroups. (B) Prevalence of hyperparathyroidism (PTH >65 pg/ml) by serum 25(OH)-vitamin D subgroups.

odds of being prediabetic or diabetic ($p=0.053$, aOR 1.50, 95% CI: 0.995, 2.256) with vitamin D deficiency.

Discussion

Consistent with the published literature, vitamin D insufficiency and deficiency were highly prevalent (combined prevalence 83%) in our cohort, and black race/ethnicity increased the risk of vitamin D deficiency, while current use of MVI and vitamin D supplements decreased the risk.^{13,14,16,21} Older age is traditionally a risk factor for vitamin D deficiency; however, we found younger age was associated with vitamin D deficiency, which was also reported in the SUN cohort.¹⁶ In our study, this finding may be partly explained by higher rates of MVI use and vitamin D supplementation in patients aged ≥ 35 years, which was corroborated by a higher proportion with detectable serum vitamin D₂, the form of vitamin D associated with dietary supplementation (data not shown). There was no significant difference in race/ethnicity by age group (data not shown).

Vitamin D deficiency doubled the odds of hyperparathyroidism in our cohort, and the prevalence of hyperparathyroidism increased with severity of vitamin D deficiency.

The prevalence of hyperparathyroidism was 30% among patients with vitamin D deficiency and 23% among those with vitamin D insufficiency. Among patients with severe vitamin D deficiency, 38% had hyperparathyroidism, and severe vitamin D deficiency was associated with elevated levels of alkaline phosphatase, a marker for increased bone turnover. These findings are consistent with known biological pathways linking vitamin D deficiency with secondary hyperparathyroidism and increased bone resorption. Serum 25(OH)-vitamin D less than 30 ng/ml is associated with increased PTH; PTH activates osteoblasts, which in turn catalyzes the maturation of preosteoclasts into osteoclasts, which resorb bone by dissolving its mineralized matrix.¹⁸ The high prevalence of vitamin D deficiency in HIV-infected patients, its association with hyperparathyroidism, and its association with elevated alkaline phosphatase in the setting of severe vitamin D deficiency are concerning, as it suggests that vitamin D deficiency may exacerbate the risk for osteopenia, osteoporosis, and fractures with HIV infection and cART use. The 2011 Endocrine Society clinical practice guidelines recommend that all HIV-infected patients on cART should be given at least two to three times more vitamin D for their age group.¹ Furthermore, in HIV-infected patients with vitamin D deficiency and hyperparathyroidism, or with severe vitamin D deficiency, screening for osteomalacia (i.e., serum and urinary phosphate) and osteopenia (i.e., bone scan) and aggressive vitamin D repletion may be warranted.⁴

EFV use was associated with vitamin D deficiency in multivariate analysis, but not in univariate analysis. The higher proportion of MVI and vitamin D supplementation among patients on EFV compared with patients not on cART (39% vs. 21%, $p=0.005$) may have mitigated the effects of EFV on vitamin D deficiency in univariate analysis. Conversely, PI use was associated with decreased odds of vitamin D deficiency in univariate analysis, but not in multivariate analysis, which may be partially explained by a higher proportion of MVI and vitamin D supplementation among patients on PI (37%). We were unable to detect any significant difference in black race/ethnicity based on current cART use.

Our findings that EFV use was correlated with lower vitamin D levels, and PI use with higher vitamin D levels are consistent with the published literature.^{16,21,24,29–31} Efavirenz is thought to induce CYP24 of the cytochrome P450 system, which may lead to increased metabolism of 25(OH)-vitamin D and 1,25(OH)₂-vitamin D to inactive metabolites.^{21,32,33} Since 1,25(OH)₂-vitamin D is thought to inhibit PTH secretion, use of efavirenz may lead to an increase in PTH and hyperparathyroidism. Protease inhibitors are associated with increased 25(OH)-vitamin D levels and paradoxically with decreased BMD, and one potential mechanism may be its inhibition of 1 α -hydroxylase, an enzyme responsible for converting 25(OH)-vitamin D to the biologically active 1,25(OH)₂-vitamin D.²¹ Use of PIs may decrease conversion of 25(OH)-vitamin D to 1,25(OH)₂-vitamin D, leading to increased PTH and hyperparathyroidism, decreased calcium absorption, and increased rates of osteopenia and osteoporosis.^{18,21} However, in our study, we were not able to detect a statistical difference in rates of hyperparathyroidism among those not on cART, on EFV-based ART, or on PI-based ART. Of the nucleoside/nucleotide analogues, tenofovir has been associated with loss of BMD.^{4,21,34} Tenofovir use was associated with vitamin D deficiency in the SUN study, and two studies reported an association of tenofovir use with hyperparathyroidism in the setting of vitamin D deficiency.^{16,35,36} In contrast, we were not able to find an association between tenofovir use and either vitamin D deficiency or hyperparathyroidism.

In our study, vitamin D deficiency trended toward increased odds of being prediabetic or diabetic in HIV-infected patients, after controlling for weight. Vitamin D deficiency has been associated with glucose intolerance and insulin resistance, and the large prospective Nurse' Health Study found that vitamin D supplementation reduced the risk for type 2 diabetes.^{18,20,37–39} Although vitamin D receptors have been found on pancreatic β -islet cells, and the biologically active form of vitamin D [1,25(OH)₂-vitamin D] was shown to stimulate insulin production, the precise mechanism by which vitamin D affects insulin production and risk of diabetes remains unknown. The risk of diabetes mellitus in HIV-infected patients is unclear; it was found to be higher among HIV-infected patients in the Multicenter AIDS Cohort Study, but not in other studies.^{5,40–42} Whether and how HIV infection and vitamin D deficiency may have additive or synergistic effects on insulin production and glucose tolerance are unknown.

In our study, vitamin D deficiency was independently associated with increased odds of having an HIV viral load >200 copies/mL, after adjusting for current use of antiretroviral therapy. Data from the Women's Interagency HIV Study found a similar effect inversely, in which an undetectable HIV viral load was independently associated with lowered odds of vitamin D deficiency.³¹ Published data on the association of vitamin D deficiency with HIV disease progression are conflicting.^{16,17,29,43,44} Two large studies have reported an association of vitamin D deficiency with HIV disease progression and all-cause mortality, but not with CD4 count or viral load.^{29,44} A potential biological mechanism for the role of vitamin D deficiency in HIV disease progression may be alteration in a type of programmed cell death called autophagy. Autophagy allows for clearance of intracellular organisms in innate immunity and assists with antigen presentation in adaptive immunity.⁴⁵ The

biologically active form of vitamin D [1,25(OH)₂-vitamin D] induces autophagy in cells by binding to the vitamin D receptor, which initiates a molecular cascade that eventually leads to fusion of autophagosomes with lysosomes and clearance of intracellular organisms.⁴⁵ Because HIV infection alters and blocks autophagy in infected macrophages and lymphocytes as a mechanism to increase intracellular HIV viral replication,⁴⁵ vitamin D deficiency may augment HIV viral replication by further dampening autophagy in infected cells, and, hence, increase plasma viral load and worsen HIV disease progression.

This study has several limitations. We were unable to account for the degree of outdoors exposure to sunlight in our patients, although most had vitamin D levels measured during the winter season. We were unable to account for the body mass index (BMI) in our patients. Low BMI in HIV-infected patients is known to be a risk factor for vitamin D deficiency and osteopenia,¹⁶ and high BMI is associated with insulin resistance. Data on gamma-glutamyltransferase (GGT) to assess whether the elevated alkaline phosphatase was of liver origin rather than bone was not available, and most patients did not have bone scan results available. HbA1c levels may have underestimated the prevalence of prediabetes and of diabetes in our study due to the effects of HIV infection and medication on the sensitivity of HbA1c as a measure of glycemic control.^{46–49} Although we assessed for risk factors and complications associated with vitamin D deficiency, this analysis was based on cross-sectional data, and correlation does not imply a causal relationship.

In summary, vitamin D deficiency was highly prevalent in our clinic cohort of HIV-infected adults, and was associated with efavirenz use as well as known risk factors for vitamin D deficiency. Use of protease inhibitors, MVI, and vitamin D supplementation reduced the odds of vitamin D deficiency. The prevalence of hyperparathyroidism was 30% among patients with vitamin D deficiency and 23% among those with vitamin D insufficiency, and vitamin D deficiency was associated with increased odds of hyperparathyroidism. Severe vitamin D deficiency was associated with elevated levels of alkaline phosphatase, a marker for increased bone turnover. In light of the increased risk of osteopenia in HIV-infected patients on cART, vitamin D supplementation for all HIV-infected patients on cART should be prescribed in accordance with the 2011 Endocrine Society guidelines.¹ Screening for osteomalacia and osteopenia in HIV-infected patients with severe vitamin D deficiency or hyperparathyroidism may be warranted. In our cohort of HIV-infected patients, vitamin D deficiency was associated with increased odds of being prediabetic or diabetic, and with increased odds of having an unsuppressed viral load. These findings corroborate the limited amount of published literature supporting the role of vitamin D in glucose control, immune function, and HIV disease progression. Further studies are needed to better characterize the spectrum and severity of complications associated with vitamin D deficiency in HIV infection.

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