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High 25-hydroxyvitamin D is associated with unexpectedly high plasma inflammatory markers in HIV patients on antiretroviral therapy

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Abstract

Inflammation associated with low 25-hydroxyvitamin D (25(OH)D) is associated with increased morbidity and mortality among HIV-infected patients with vitamin D deficiency. We investigated the association between 25(OH)D and soluble biomarkers among HIV-infected patients on stable antiretroviral therapy. This is a cross-sectional study. This study focuses on assessment in subjects 40 years or older on stable antiretroviral therapy (ART) for >3 months. Chemiluminescent immunoassay was used to determine plasma 25(OH)D levels. Plasma soluble biomarkers were measured by Luminex technology. Multivariable linear regression analysis was used to assess the associations between $\log_{10}$-25(OH)D and soluble biomarkers.

Of 138 patients, median age was 50.5 (45, 57) years and 25(OH)D was 34.0 (25.0, 42.3) ng/mL. The majority were males (88%) and had undetectable HIV RNA (84.8%); 19 (13.8%) had 25(OH)D ≥50 ng/mL. Spline regression analyses suggested a J-shaped relationship between various plasma biomarkers and 25(OH)D. Among subjects with 25(OH)D ≥20 ng/mL, multivariable linear regression showed positive association between 25(OH)D and interleukin (IL)-10 (β = 1.84, P < 0.001), IL-6 (β = 0.72, P = 0.02), MPO (β = 0.47, P = 0.02), serum amyloid A (β = 1.20, P = 0.04), and tumor necrosis factor (TNF)-α (β = 0.51, P = 0.04). High 25(OH)D (≥50 ng/mL) was associated with higher IL-6 (β = 0.30, P = 0.009), IL-8 (β = 0.14, = 0.005), IL-10 (β = 0.43, P = 0.02), and TNF-α (β = 0.20, P = 0.04), independent of age, sex, ethnicity, body mass index, hepatitis C co-infection, current smoking status, CD4%, and HIV RNA.

In older HIV-infected patients, high 25(OH)D was associated with higher (not lower) levels of proinflammatory cytokines. Higher-than-optimal 25(OH)D may be associated with immune dysregulation and may pose a potential health risk among HIV-infected patients.

Abbreviations: 25(OH)D = 25-hydroxyvitamin D, CAC = coronary artery calcium, cIMT = carotid intima media thickness, CRP = C-Reactive Protein, FMD = flow mediated dilatation, HAHC-CVD = Hawaii Aging with HIV Cardiovascular Disease cohort, IFN-γ = interferon-gamma, IL = interleukin, MCP-1 = monocyte chemoattractant protein-1, MMP-9 = matrix metallopeptidase-9, MPO = myeloperoxidase, PAI-1 = plasminogen activator inhibitor type 1, SAA = serum amyloid A, SAP = serum amyloid P, sE-selectin = soluble E-selectin, sICAM-1 = soluble intercellular adhesion molecule-1, sVCAM-1 = soluble vascular cell adhesion molecule-1, TNF-α = tumor necrosis factor-alpha, VEGF = vascular endothelial growth factor.

Keywords: cytokines, Hawaii, HIV, hypervitaminosis D, inflammation, TNF-alpha, vitamin D

1. Introduction

High rates of low 25-hydroxyvitamin D (25(OH)D) have been reported among HIV-infected patients. This increased prevalence is multifactorial, involving traditional factors for 25(OH)D deficiency, metabolic complications associated with HIV, and pharmacologic effect of antiretroviral therapy.[1] Low 25(OH)D has been associated with increased morbidity and mortality among

HIV-infected patients and is thought to be secondary to increased proinflammatory cytokines in the setting of low 25(OH)D levels.[2]

We investigated the association between soluble biomarkers and 25(OH)D levels in patients enrolled in the in the Hawaii Aging with HIV Cardiovascular (HAHC-CVD) cohort, a 5-year prospective study of aging HIV-infected patients on stable antiretroviral therapy. More recent data have emerged demonstrating a possible U-shaped or a reverse J-shaped association between 25(OH)D and mortality—with increased risk of death in both low and high 25(OH)D levels.[3,4] We analyzed whether higher-than-optimal 25(OH)D is associated with immune dysregulation and inflammation in the HAHC-CVD cohort.

2. Methods

2.1. Subjects and study design

This is a cross-sectional analysis of the entry data of the subjects enrolled into the HAHC-CVD cohort. Subjects had documented HIV infection, ≥40 years old, and on stable antiretroviral therapy for ≥3 months. Subjects who were recently hospitalized, had active infection, malignancy, or AIDS-defining illness at the time of enrolment were excluded. The study was approved by the Committee on Human Studies of the University of Hawaii and written informed consent was obtained from all participants. A full description of the cohort recruitment has been previously published and described elsewhere.[5]

2.2. Measuring 25-hydroxyvitamin D, soluble biomarkers, and markers of arterial dysfunction

Fasting blood samples were collected (nothing by mouth for 12 hours except water), stored in EDTA tubes, frozen at -140^0C and forwarded to LipoScience Inc. (Raleigh, NC). Chemiluminescent immunoassay (DiaSorin) was used to measure serum 25 (OH)D levels.

Milliplex Human Cardiovascular Disease panels (EMD Millipore) were used to measure the following plasma soluble biomarkers: C-reactive protein (CRP), interferon-gamma (IFN-γ), interleukin-1beta (IL-1β), IL-6, IL-8, IL-10, matrix metallopeptidase-9 (MMP-9), monocyte chemoattractant protein-1 (MCP-1), myeloperoxidase (MPO), plasminogen activator inhibitor type 1 (PAI-1), serum amyloid A (SAA), serum amyloid P component (SAP), soluble E-selectin (sE-selectin), soluble vascular cell adhesion molecule-1 (sVCAM-1), soluble intercellular adhesion molecule-1 (sICAM-1), tumor necrosis factor-alpha (TNF-α), and vascular endothelial growth factor (VEGF).

Three different markers of arterial dysfunction were available in the database: carotid intima media thickness (cIMT), flow-mediated dilatation (FMD), and coronary artery calcium (CAC). Methods utilized to obtain cIMT, FMD, and CAC have been previously published.[5]

2.3. Statistical analyses

Simple and multivariable linear regression analyses were performed to identify whether 25(OH)D was associated with soluble biomarker levels. Multiple linear regression models were adjusted for the following variables that may affect cytokine levels: age, sex, white ethnicity, body mass index (BMI), hepatitis C co-infection, current smoking status, undetectable HIV RNA, and CD4 percent. Soluble biomarkers and 25(OH)D levels were log-transformed to improve normality of residuals. To investigate any possible nonlinear associations, spline regression analyses were performed and quadratic functions were fitted in the models.

Hawaii is located in the tropics (latitude 21 degree North) and has 2 seasons, summer (May 1–September 30) and winter (October 1–April 30). We have previously reported that seasonal variations in 25(OH)D levels in the HAHC-CVD cohort exist.[6] In brief, median 25(OH)D levels were higher among subjects enrolled in summer compared to winter (36.9 [25.0, 44.5] ng/mL vs. 29.6 [22.0, 38.0] ng/mL, $P = 0.01$). However, the association between season and 25(OH)D was not preserved in the final multivariate regression models when adjusted for other factors affecting 25(OH)D levels, such as BMI and ethnicity. Hence, season was not included in the regression models of the present study. Statistical analyses were performed using SAS 9.4 (SAS Institute, Cary, NC, 2002–2012).

3. Results

Of 138 subjects, median 25(OH)D (quartile 1, quartile 3) was 34.0 (25.0, 42.3) ng/mL. When grouped by 25(OH)D levels, 15 (10.9%) had 25(OH)D <20 ng/mL, 42 (30.4%) had levels between 20 and 30 ng/mL, and 81 (58.7%) had 25(OH)D >30 ng/mL, of which, nineteen (13.7%) subjects had 25(OH)D ≥50 ng/mL. None of the subjects had 25(OH)D <10 ng/mL. Three subjects (1.9%) were on calcium and vitamin D supplements.

No significant differences in age (50.5 [45, 57] years), sex (88% male), proportion of subjects with history of HCV (9%), current smokers (21.7%), percentage of undetectable HIV RNA (84.8%), current CD4 count (490 [341, 635] cells/μL) and CD4% (30 [21,37]) were found between 25(OH)D groups. BMI was significantly different between the 3 groups: 25.5 (24.6, 27.3), 26.7 (24.5, 31.4), 25.3 (23.1, 27.4) for the 3 groups, respectively ($P = 0.02$).

Spline regression analyses suggested a nonlinear association between various plasma biomarkers and 25(OH)D. Using scatter plot with a fitted quadratic function, a J-shaped association between 25(OH)D and soluble biomarkers was found (Figure 1). To further investigate this association, subjects with 25(OH)D ≥20 ng/mL were separately analyzed.

Among subjects with 25(OH)D ≥20 ng/mL, univariable linear regression showed a positive association between 25(OH)D and the following soluble biomarkers: IL-10 (β=1.7, $P < 0.0001$), IL-6 (β=0.55, $P = 0.04$), MPO (β=0.31, $P = 0.10$), sICAM (β= 0.22, $P = 0.06$), SAA (β=0.91, $P = 0.08$), sVCAM (β=0.15, $P = 0.06$), and TNF-α (β=0.61, $P = 0.008$). In the multivariable regression models, 25(OH)D remained associated with IL-10 (β=1.84, $P < 0.001$), IL-6 (β=0.72, $P = 0.02$), MPO (β=0.48, $P = 0.03$), SAA (β=1.21, $P = 0.04$), and TNF-α (β=0.51, $P = 0.04$). When 25(OH)D was dichotomized using the cutoff of 25 (OH)D ≥50 ng/mL, high 25(OH)D was associated with higher IL-6, IL-8, IL-10, and TNF-α in multiple linear regression analyses (Table 1).

Interestingly, when analysis was restricted among subjects with low 25(OH)D (<20 ng/mL), univariable linear regression analysis showed that lower 25(OH)D was associated with higher cytokines, consistent with previously published studies (albeit not statistically significant): selectin (β=−1.65, $P = 0.14$), sVCAM (β=−1.06, $P = 0.08$), sICAM (β=−2.061, $P = 0.07$), MPO (β=−2.341, $P = 0.07$), tissue plasminogen activator inhibitor-1 (β=−0.36, $P = 0.73$), CRP (β=−0.002, $P = 0.99$), IL-10, (β=−2.18, $P = 0.24$), TNF-α (β=−0.09, $P = 0.95$), and MCP-1 (β=−0.16, $P = 0.74$).

Markers of arterial dysfunction as assessed by cIMT and FMD were similar between subjects with 25(OH)D <20 ng/mL, 20 to
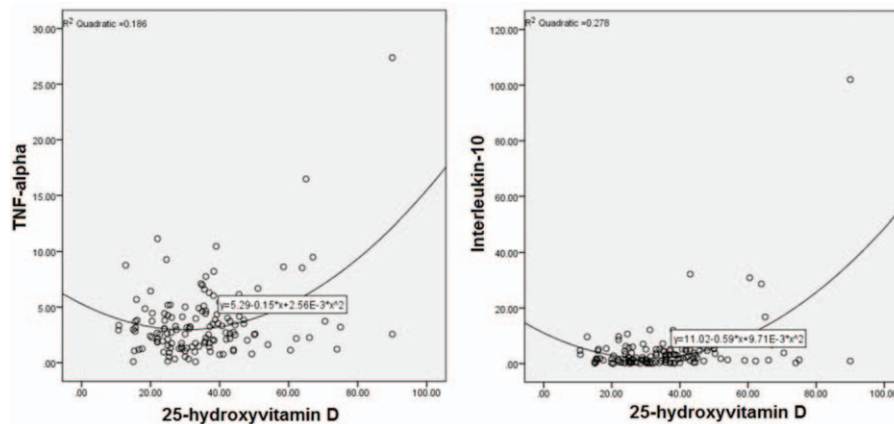
Figure 1. Scatter plot demonstrating a J-shaped relationship between 25-hydroxyvitamin D and tumor necrosis factor (TNF)-alpha and interleukin (IL)-10 after fitting a quadratic function. 25-hydroxyvitamin D levels in ng/mL, whereas TNF-alpha and IL-10 levels presented in pg/mL.

50 ng/mL, and ≥50 ng/mL. CAC was present in 47% of the 138 subjects. High 25(OH)D (≥50 ng/mL) was not associated with cIMT and FMD using linear regression analyses. Similarly, 25 (OH)D ≥50 ng/mL did not predict CAC presence in logistic regression analyses.

4. Discussion

Low 25(OH)D is associated with occurrence of both AIDS and non-AIDS-defining events, disease progression, and increased mortality.[7] The Endocrine Society considers 25(OH)D ≥30 ng/mL as the optimal level for bone health and muscle function, but the upper limit of normal remains controversial.[8]

The detrimental effects of low 25(OH)D is thought to be from the inflammation associated with low vitamin D levels. In HIV-infected individuals, a recent publication by Hoffman et al showed that high 25(OH)D was associated with lower TNF-α.[9] Similarly, among subjects with TB-HIV co-infection, severe 25 (OH)D deficiency (<10 ng/mL) was associated with higher levels of TNF, IL-6, and IL-8.[10] In a subset of the EuroSIDA cohort, 25 (OH)D <10 ng/mL was associated with increased hsIL-6.[7] Among 322 HIV-infected subjects in Nepal, 25(OH)D <20 ng/mL was associated with higher CRP levels.[11] All four studies show association between low 25(OH)D and higher proin-flammatory markers. However, all 4 cohorts had low proportion of patients with 25(OH)D> 50 ng/mL. It is likely that these

Table 1

25-hydroxyvitamin D as predictor of soluble plasma biomarkers assessed using multiple linear regression models.

Dependent variable[*]	25 (OH)D as continuous variable		High 25 (OH)D (>50 ng/mL)	
	Regression coefficient	P	Regression coefficient	P
CRP	0.52	0.34	0.32	0.12
IFN-γ	0.09	0.76	−0.04	0.69
IL-10	1.84	<0.001[**]	0.44	0.02[**]
IL-1β	0.08	0.50	0.04	0.41
IL-6	0.72	0.02[**]	0.30	0.009[**]
IL-8	0.28	0.04[**]	0.14	0.005[**]
MCP-1	0.16	0.19	0.08	0.06
MMP-9	−0.02	0.89	0.01	0.82
MPO	0.48	0.03[**]	0.13	0.09
SAA	1.21	0.04[**]	0.38	0.07
SAP	0.21	0.48	0.01	0.93
sE-Selectin	0.04	0.82	0.12	0.07
sICAM-1	0.12	0.33	0.05	0.29
sVCAM-1	0.14	0.09	0.01	0.65
TNF-α	0.51	0.04[**]	0.20	0.04[**]
tPAI-1	−0.18	0.15	−0.04	0.40
VEGF	0.18	0.57	0.01	0.92

Analyses included subjects with 25(OH)D >20 ng/mL. 25(OH)D, 25-hydroxyvitamin D; CRP, C-reactive protein; IFN, interferon; IL, interleukin; MCP-1, monocyte chemoattractant protein-1; MMP-9, matrix metallopeptidase-9; sICAM, soluble intercellular adhesion molecule-1; sVCAM, soluble vascular cell adhesion molecule-1; TNF, tumor necrosis factor; tPAI-1, tissue plasminogen activator inhibitor-1; VEGF, vascular endothelial growth factor.

[*] Soluble biomarker and 25(OH)D levels were log-transformed to improve normality. Separate multiple linear regression models were conducted with the listed soluble biomarkers as dependent variable and 25 (OH)D as independent variable. Linear regression models were adjusted for the following variables that may affect cytokine levels: age, sex, white ethnicity, body mass index, hepatitis C co-infection, current smoking status, HIV RNA level, and CD4 percent.

[**] Statistically significant.

previous studies were underpowered to detect the association between high 25(OH)D levels and cytokines because of relatively low number of patients with normal/high 25(OH)D. Furthermore, because 25(OH)D deficiency is prevalent among HIV patients, there are limited data on the detrimental effects of high vitamin D in this population.

Vitamin D toxicity is usually seen in levels >750 nmol/L (300 ng/mL) and typically occurs in acute vitamin D intoxication/ingestion. Patients present with hypercalcemia, anorexia, nausea and vomiting, and renal failure. The exact molecular processes of vitamin D toxicity remain to be elucidated. One theory suggests that increased levels of vitamin D metabolites (including vitamin D_3; $25(OH)D_3$; $24,25(OH)_2D_3$; $25,26(OH)_2D_3$; and $25(OH)D_3$-26,23-lactone) saturate the vitamin D-binding proteins in the bloodstream causing an increase in the free concentrations of $1\alpha,25(OH)_2D_3$ and $25(OH)D_3$. This increase in total 25(OH) and free $1\alpha,25(OH)_2D_3$ is implicated as the triggering events for toxicity, resulting in hypercalcemia and hyperphosphatemia.[12,13]

To prevent detrimental effects of high vitamin D, some authors suggest using 100 ng/mL as the higher limit of normal 25(OH)D, but a recent study demonstrated increased mortality in 25(OH)D levels as low as 50 ng/mL.[3] Data from vitamin D supplementation studies have shown that for serum 25(OH)D concentrations to exceed 100 nmol/L (40 ng/mL), total vitamin D supply of 4000 IU/day must be met. Adverse effects are typically seen beyond a total vitamin D supply of 250 µg (10,000 IU)/day.[14] In our cohort, we found a surprising high 13.7% (19 subjects) had 25 (OH)D >50 ng/mL. Although only 3 of these 19 subjects acknowledged vitamin D supplementation, it is highly possible that these high levels were from exogenous use.

Issues of vitamin D use was not the primary objective of the HAHC-CVD cohort and it is possible that more intensive queries into use of over-the-counter supplemental medications may have uncovered more evidence of vitamin D supplementation.

We found that levels >50 ng/mL are associated with higher levels of inflammatory markers, even after adjusting for traditional factors that may affect cytokine levels. Although we did not find any direct suggestion that levels >50 ng/mL was associated with markers of arterial injury, this relationship between high 25(OH)D levels and higher levels of inflammatory markers is of potential clinical concern and warrants further investigation in a larger cohort.

Interestingly, consistent with previous studies, lower levels of 25(OH)D showed a trend toward higher inflammatory markers in our cohort (albeit not statistically significant). This may further suggest that a J-shaped association between vitamin D and cytokines exist—with increased inflammation in both extremely high and low 25(OH)D. We do not have any subjects with 25 (OH)D <10 ng/mL, which limited the analysis of the association of low 25(OH)D and soluble biomarkers.

This study is limited by its cross-sectional nature, the small number of subjects in the dataset, the retrospective nature of the study, and likely by incomplete clinical data on vitamin D supplementation.

In summary, we report for the first time that high 25(OH)D is associated with higher proinflammatory markers. Our data suggest that there may be dangers to indiscriminate over-supplementation with vitamin D, and that perhaps periodic monitoring of 25(OH)D levels may be prudent to ensure that patients' 25(OH)D do not go beyond optimal levels. More research is warranted to elucidate the implication of high 25(OH)D levels and higher levels of plasma biomarkers.

Acknowledgments

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