Short communication

Association between initiation of antiretroviral therapy with efavirenz and decreases in 25-hydroxyvitamin D

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Background: We aimed to determine whether antiretroviral therapy (ART) initiation with efavirenz (EFV) is associated with decreases in 25-hydroxyvitamin D (25[OH]D) compared with non-EFV regimens.
Methods: 25(OH)D was measured from stored plasma samples in 87 ART-naive HIV-positive patients prior to ART initiation with EFV-containing (*n*=51) or non-EFV-containing (89% with protease inhibitors; *n*=36) regimens from a single clinic in Cleveland (OH, USA). A repeat measurement was made 6–12 months after ART initiation. The change in 25(OH)D after ART initiation and the prevalence of patients with hypovitaminosis D (≤37.5 nmol/l [15 ng/ml]) after ART initiation was compared in those who initiated ART with and without EFV using multivariable linear and modified Poisson regression, respectively.
Results: Prior to ART initiation, the median (interquartile range [IQR]) 25(OH)D concentration was 52.7 nmol/l (IQR 32.2–72.1), with 33% prevalence of hypovitaminosis D. After 6–12 months of ART, 25(OH)D decreased by a mean ±SE of -12.7 ±3.7 nmol/l in the EFV group relative to the non-EFV group (*P*=0.001) after adjustment for baseline 25(OH)D concentration, race and season (non-summer versus summer) at the visit 6–12 months after ART initiation. Similarly, after multivariable adjustment, the risk of hypovitaminosis D after ART initiation was significantly higher in the EFV group compared with the non-EFV group (prevalence ratio 1.8 [95% confidence interval 1.2–2.8]; *P*=0.007).
Conclusions: ART initiation with EFV is associated with significant decreases in 25(OH)D and an increased risk of hypovitaminosis D compared with non-EFV regimens.

Introduction

Vitamin D deficiency is common among HIV-infected patients [1,2], is correlated with reduced bone mineral density (BMD) [3] and might contribute to the higher prevalence of osteoporosis [4] and fragility fractures [5] in HIV-infected patients compared with HIV-uninfected controls. Recent cross-sectional studies have described low 25-hydroxyvitamin D (25[OH]D) concentrations in non-nucleoside reverse transcriptase inhibitor (NNRTI)-treated patients [1,2], particularly those treated with efavirenz (EFV) [6–8]. Our goal was to determine whether the change in 25(OH)D concentrations differs between antiretroviral therapy (ART)-naive HIV-infected patients initiating EFV-containing regimens compared with those initiating non-EFV regimens.

Methods

Study patients

Study patients were enrolled from a clinical cohort at the Center for AIDS Research (CFAR) at the Case Western Reserve University (Cleveland, OH, USA) and were identified for a study that examined bone turnover with ART initiation [9]. Eligible patients were HIV-infected adults, aged 18–50 years, who had initiated ART and who had a stored plasma sample prior to and within 6–12 months of ART initiation. Exclusion criteria were known osteoporosis, fragility fractures or prior therapy with bisphosphonates or other bone therapies. Demographic and clinical data were extracted from the CFAR database and from the clinical charts. Each patient provided signed written informed consent that was approved by the Institutional Review Board of University Hospitals Case Medical Center (Cleveland, OH, USA).

Laboratory assays

Pretreatment and on-treatment plasma samples were stored at -80°C. 25(OH)D was measured using radioimmunoassay (DiaSorin, Stillwater, MN, USA) in the Advanced Chemistry Laboratory (Johns Hopkins

University, Baltimore, MD, USA). The coefficients of variation for these assays were 5.2% (intraassay) and 7.9% (interassay). Hypovitaminosis D was defined as ≤37.5 nmol/l (15 ng/ml), as in previous epidemiological investigations [10,11].

Statistical analyses

Descriptive characteristics of the EFV group and the non-EFV group were compared using Wilcoxon rank-sum and χ^2 tests as appropriate. Determinants of hypovitaminosis D prior to ART initiation were assessed using modified Poisson regression with robust variance estimates [12]. Covariates that were significantly associated with 25(OH)D were evaluated in the final multivariable model. The change in 25(OH)D prior to and 6–12 months after ART initiation was compared between the EFV and non-EFV groups using both parametric (Student's *t*-test) and non-parametric (Wilcoxon rank-sum test) methods. Multiple linear regression was used to assess the difference in the change of 25(OH)D after ART initiation attributable to EFV after adjustment for baseline 25(OH)D concentrations, race (non-White versus White) and season at the visit 6–12 months after ART initiation. Because summer 25(OH)D concentrations tended to be higher than 25(OH)D concentrations during the other seasons, the season variable was collapsed to non-summer versus summer. Multivariable Poisson regression was used to estimate prevalence ratios of hypovitaminosis D in EFV- versus non-EFV-treated patients 6–12 months after ART initiation, after adjustment for potential confounding variables. Two-sided *P*-values <0.05 were considered as significant. Analyses were performed using STATA 10.1 (Stata Corporation, College Station, TX, USA).

Results

Baseline characteristics of the cohort

HIV-infected patients who initiated ART with EFV (*n*=51) and non-EFV regimens (*n*=36) were similar with respect to demographic and disease variables (Table 1). Known HIV duration was longer among those receiving non-EFV regimens (*P*=0.03).

Among those who initiated ART with non-EFV regimens, 89% (32/36) initiated with a protease inhibitor (PI). Of these 32 PI-treated patients, 31 also received low-dose ritonavir. Nucleoside combination therapy was used in 4 of 36 patients in the non-EFV group. Zidovudine (AZT) use was lower (31% versus 57%; *P*=0.02) and tenofovir disoproxil fumarate (TDF) use tended to be higher (61% versus 43%; *P*=0.1) in the non-EFV group compared with the EFV group.

A total of seven patients changed ART during the observation period. Of the 51 who began on EFV, 2 changed to a non-EFV-containing regimen. In another participant, AZT was discontinued from a regimen that contained abacavir/lamivudine/EFV. Of the 36 who began ART with non-EFV regimens, 2 switched to an EFV-containing regimen, 2 changed the specific PI used and 1 switched AZT for TDF. One participant in the EFV group did not have a repeat 25(OH)D determination after ART initiation and was therefore excluded from the longitudinal analysis.

25(OH)D prior to ART initiation

Prior to ART initiation, 33% (*n*=29) had hypovitaminosis D. White patients had higher mean ±SD 25(OH)D concentrations than non-White patients (76.9 ±29.2 versus 42.2 ±20.7 nmol/l; *P*<0.0001). The 25(OH)D concentrations were higher in the summer compared with non-summer (69.4 ±27.5 versus 49.6 ±27.4 nmol/l; *P*=0.003). Longer known duration of HIV infection tended to be associated with lower 25(OH)D (β=-1.3 nmol/l per year; *P*=0.067). Age, sex, nadir CD4$^+$ T-cell count, body weight and pretreatment HIV RNA were not correlated with pretreatment 25(OH)D. In a multivariable analysis, baseline hypovitaminosis D was associated with non-White race with a prevalence ratio [PR] of 6.7 (95% confidence interval [CI] 1.7–25.6; *P*=0.006), season (non-summer versus summer) with a PR of 4.6 (95% CI 1.2–17.8; *P*=0.03) and known HIV duration with a PR of 1.06 per year (95% CI 1.02–11.09; *P*=0.003).

Efavirenz use and changes in 25(OH)D with ART initiation

After ART initiation, the median (interquartile range [IQR]) change in 25(OH)D was -12.7 nmol/l (-20.7–2.7) in the EFV group and 1.0 nmol/l (-10.2–14.5) in the non-EFV group (*P*=0.004), corresponding to a percentage change of -20% (-38–11) in the EFV group and 2% (-15–46) in the non-EFV group (*P*=0.004). Figure 1 shows the mean (±SD) change in 25(OH)D concentration with ART initiation in the EFV and non-EFV groups. In a multivariate linear regression model, the mean ±SE change in the EFV group was -12.7 ±3.7 nmol/l lower than in the non-EFV group, after adjustment for baseline 25(OH)D concentration, race and season at the visit after ART initiation. On-treatment CD4$^+$ T-cell count, ART duration, known HIV duration or the choice of nucleoside/nucleotide backbone were not associated with the change in 25(OH)D (TTB, data not shown). Similar results were obtained when the analysis was restricted to the 79 participants who remained on the same treatment throughout the study interval.

After ART initiation, 48% (24/51) in the EFV group and 31% (11/36) in the non-EFV group had hypovitaminosis D (*P*=0.1). After adjustment for baseline 25(OH)D concentration, race and season at the visit after ART initiation, those in the EFV group had an

Table 1. Characteristics of patients before and after antiretroviral initiation

Characteristic	EFV-treated (*n*=51)	Non-EFV-treated (*n*=36)	*P*-value
Pretreatment			
Age, years	35 (30–40)	35 (30–45)	0.53
Non-White race, *n* (%)	31 (61)	24 (67)	0.58
Male gender, *n* (%)	41 (80)	24 (67)	0.15
Weight, kg	74.8 (66.4–87.5)	74.4 (64.2–84.0)	0.55
Nadir CD4$^+$ T-cell count, cells/mm^3	194 (53–259)	140 (20–253)	0.19
Baseline HIV RNA, $\log_{10}$ copies/ml	4.9 (4.5–5.1)	4.9 (4.6–5.3)	0.82
Known HIV duration, months	11 (2–32)	24 (6–82)	0.03
Season			0.96
Spring, *n* (%)	12 (24)	9 (25)	
Summer, *n* (%)	15 (29)	9 (25)	
Autumn, *n* (%)	13 (26)	9 (25)	
Winter, *n* (%)	11 (22)	9 (25)	
25(OH)D, nmol/l	52.7 (34.9–68.1)	53.7 (30.7–77.4)	0.89
25(OH)D≤37.5 nmol/l, *n* (%)	14 (27)	15 (42)	0.17
On-treatment			
Protease inhibitor, *n* (%)	0 (0)	32 (89)	–
Lopinavir, *n* (%)	0 (0)	5 (14)	–
Atazanavir, *n* (%)	0 (0)	26 (72)	–
Fosamprenavir, *n* (%)	0 (0)	1 (3)	–
Tenofovir disoproxil fumarate, *n* (%)	22 (43)	22 (61)	0.10
Zidovudine, *n* (%)	29 (57)	11 (31)	0.02
Abacavir, *n* (%)	10 (20)	7 (19)	0.98
ART duration, days	252 (205,294)	243 (217,288)	0.88
HIV RNA<400 copies/ml, *n* (%)	50 (98)	34 (94)	0.37
CD4$^+$ T-cell count, cells/mm^3	398 (209–528)	409 (275–553)	0.62
Season of second sample			0.27
Spring, *n* (%)	16 (31)	6 (17)	
Summer, *n* (%)	15 (29)	13 (36)	
Autumn, *n* (%)	9 (18)	11 (31)	
Winter, *n* (%)	11 (22)	6 (17)	
25(OH)D, nmol/l	37.9 (26.5–59.9)[a]	62.2 (32.9–75.9)	0.05
25(OH)D≤37.5 nmol/l, *n* (%)	24 (48)[a]	11 (31)	0.10

Values represent median (interquartile range) unless indicated otherwise. [a]*n*=50 because second 25-hydroxyvitamin D (25[OH]D) sample was unavailable for one efavirenz (EFV)-treated patient. ART, antiretroviral therapy.

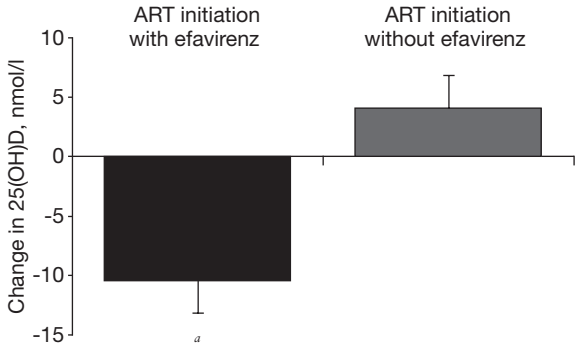
increased prevalence of hypovitaminosis D after ART initiation compared with the non-EFV group (PR 1.8 [95% CI 1.2–2.8]; *P*=0.007). Similar results were obtained when hypoviatminosis D was defined as <50 nmol/; (20 ng/ml; TTB, data not shown).

Discussion

In this longitudinal study of HIV-infected patients initiating ART, we found that treatment with EFV was associated with a significant decrease in 25(OH) D after 6–12 months compared with ART initiation with non-EFV regimens. Similarly, the risk of hypovitaminosis D 6–12 months after ART initiation was significantly higher in EFV-treated patients compared with non-EFV-treated patients.

Previous cross-sectional studies have suggested that NNRTI use is associated with abnormal vitamin D

Figure 1. Mean change in 25(OH)D 6–12 months after initiating ART with and without efavirenz



Error bars represent ±SE. [a]*P*=0.0003 within group change and *P*=0.002 for between-group difference. ART, antiretroviral therapy; 25(OH)D, 25-hydroxyvitamin D.

metabolism [1,2]. Most recently, a large cross-sectional study from a single London (UK) clinic showed that the odds of low vitamin D levels was 90% higher in EFV-treated patients compared with non-EFV-treated patients [8], and two case reports have implicated EFV as the cause of severe vitamin D deficiency and osteomalacia [6,7]. Our study extends these observations, demonstrating in a prospective study that the initiation of EFV is associated with decreases of 25(OH)D compared with non-EFV regimens.

The mechanisms underlying the effect of EFV on vitamin D metabolism require clarification. Vitamin D is either made in the skin or is derived from ingested sources and is then converted to 25(OH)D, the major circulating metabolite. 25(OH)D is then either converted to its active form, $1,25(OH)_2D$, by 1-α hydroxylase or is transformed to its inactive metabolite (calcitroic acid) through the cytochrome P450 enzyme, 24-hydroxylase (CYP24). EFV is a potent inducer of cytochrome P450 enzymes [13] and has recently been shown in an *in vitro* model to induce the expression of CYP24 [14]; therefore, similar to the mechanism of antiepileptic drugs on vitamin D metabolism [15], EFV might lower 25(OH)D concentrations by increasing the metabolism of 25(OH)D into calcitroic acid. It is not known whether certain polymorphisms in genes related to vitamin D metabolism or EFV pharmacokinetics might confer increased susceptibility to EFV-induced vitamin D deficiency, but this is an important area of future investigation.

The clinical significance of the effect of EFV on vitamin D metabolism is not clear. In the general population, vitamin D deficiency has been associated with lower BMD and fracture [16,17], as well as a variety of non-skeletal outcomes, including incident colon, prostate and breast cancer [18–21], insulin resistance [22], muscle weakness and risk of falling [23], and cardiovascular disease [24]. As a result of these observations, most experts recommend maintaining 25(OH)D concentrations >74.9–79.9 nmol/l (30–32 ng/ml) for optimal skeletal and non-skeletal health [25,26], and concentrations <37.5–50 nmol/l (15–20 ng/ml) are associated with increases in parathyroid hormone in various populations [27–31], suggesting physiologically important vitamin D deficiency. We defined hypovitaminosis D as <37.5 nmol/l and found that EFV use was associated with an 80% increased prevalence compared with non-EFV-containing regimens. Our study was limited by lack of parathyroid hormone or BMD measurements to assess the effect of EFV-induced vitamin D deficiency on bone health. Further studies are needed to understand the consequences of vitamin D deficiency on health outcomes in HIV-infected patients and the role of EFV.

Our study had additional limitations. First, the use of EFV and non-EFV regimens was not randomized and, as a result, unmeasured confounders might have influenced the results. Because some factors, such as, HIV infection duration and the baseline prevalence of hypovitaminosis D, were different between the groups, all results were adjusted for baseline 25(OH)D levels, which served to minimize the influence of pretreatment differences between the groups on subsequent vitamin D levels. Second, the non-EFV group was heterogeneous in the types of ART received, although most were receiving atazanavir with boosting doses of ritonavir. *In vitro*, the PI, ritonavir, has been shown to affect vitamin D metabolism at multiple steps, including the inhibition of 1-α hydroxylase, 24-hydroxylase and 25-hydroxylase, all of which could potentially influence 25(OH)D levels, reducing both production and degradation [32]. It is unclear whether low-dose ritonavir has similar effects *in vivo* and whether other widely used PIs, such as atazanavir, have a similar effect on vitamin D metabolism. Our results suggest that non-EFV regimens are not associated with changes in 25(OH)D, but comparisons of specific PI regimens to EFV are needed, preferably with randomized treatment allocation.

In conclusion, given the high prevalence of vitamin D deficiency in HIV-infected populations, laboratory assessment of 25(OH)D and vitamin D replacement should be considered in HIV-infected patients, particularly those with additional risk factors, including EFV use.

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Disclosure statement

The authors declare no competing interests.

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