Cholecalciferol Supplementation in HIV-Infected Youth with Vitamin D Insufficiency: Effects on Vitamin D Status and T-Cell Phenotype: A Randomized Controlled Trial

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Objectives: In addition to its known effects on bone metabolism, vitamin D may regulate immune function. **Design:** We performed a randomized controlled trial (RCT) to test whether cholecalciferol supplementation can improve vitamin D status and affect the T-cell phenotype in HIV-infected youth with vitamin D insufficiency. **Methods:** Fifty-two HIV-infected patients aged 8 to 26 years and with serum 25(OH)D <30 ng/mL were randomized to receive orally vitamin D3 100,000 IU or placebo every 3 months for 4 doses. Serum 25(OH)D, $1,25(OH)_2D$, PTH, and CD4+ T cells were assessed 3 months before baseline and at 0, 3, 6, 9, and 12 months, while Th1-, Th2-, Th17-, and Treg-subsets and T-lymphocyte vitamin D receptor were assessed at 0, 3, and 12 months. **Results:** Forty-eight subjects (25 receiving vitamin D and 23 receiving placebo) completed the RCT. Cholecalciferol supplementation produced an early (3 months) decrease in PTH, a concomitant increase in 25(OH)D, and a later (6 months) increase in $1,25(OH)_2D$ levels, all persisting at 12 months. The frequency of vitamin D insufficiency at 12 months was 20% versus 60% in the intervention versus placebo group (P = .007). Cholecalciferol supplementation had no effect on CD4+ T-cell counts but was associated with a decreased Th17:Treg ratio at 3 months. **Conclusions:** In our cohort of HIV-infected youth, a 12-month cholecalciferol supplementation increased 25(OH)D and $1–25(OH)_2D$ and decreased PTH levels but had no effect on CD4+ T-cells. However, it was associated with changes in CD4+ T-cell phenotype, warranting further investigation. **Key words:** *adolescents, children, HIV, immunity, vitamin D*

There is increasing evidence that hypovitaminosis D is common in the general population.[1,2] Low dietary intake of vitamin D and reduced exposure to sunlight are probably the major risk factors. However many facets of this condition are still unclear, and recommendations for correction of vitamin D deficiency vary widely.[3–12]

A high prevalence of hypovitaminosis D has been described in HIV-infected adults[13,14] and children.[15,16] HIV infection itself and antiretroviral (ARV) treatment may be responsible for alteration of vitamin D metabolism.[17,18] Studies have shown a significant decrease in serum 25-hydroxyvitamin-D [25(OH)D] concentration in adults receiving non-nucleoside reverse transcriptase inhibitors (NNRTIs)[19,20]; however, a sure interference of ARV treatment on vitamin D metabolism has not been confirmed in more recent studies.[21,22] Because of the importance of vitamin D in bone health and the greater risk for bone disease in HIV-infected subjects, randomized controlled trials (RCTs) have been performed to test whether vitamin D

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supplementation can improve vitamin D status and bone mineral metabolism in HIV-infected children and adolescents.[23,24]

Along with its effects on bone metabolism, vitamin D is an important modulator of the immune system. The vitamin D receptor (VDR) is found in high concentrations in activated T lymphocytes and in small amounts in monocyte/macrophage cells; B lymphocytes do not contain detectable amounts of VDR.[25]

Experimental studies have shown that the active di-hydroxylated metabolite of vitamin D [1,25$(OH)_2$D] is able to skew the T-cell compartment into a more anti-inflammatory state, with inhibition of Th1 and Th17 cells and promotion of Th2 and T regulatory (Treg) subsets.[26,27]

In the context of HIV infection, in which Th1 subpopulations inhibit viral replication,[28] any alteration of the Th1/Th2 balance would be of concern. Although all the biological effects of vitamin D are mediated by the 1,25$(OH)_2$D, the 25(OH)D needs to be routinely quantified because of its longer half-life.[29] However, HIV-infected subjects may have a defective 1α-hydroxylation of 25(OH)D.[30] Thus, it is important to evaluate the effects of vitamin D supplementation in terms of both 25(OH)D and 1,25$(OH)_2$D responses.

Our study was aimed to test the hypothesis that oral supplementation with cholecalciferol (vitamin D3) is able to increase serum 25(OH)D and 1,25$(OH)_2$D levels and to affect T-cell phenotype.

METHODS

Subjects

Vertically HIV-infected children and young adults with stable disease,[31] followed at the Department of Paediatrics – Luigi Sacco Hospital (Milan, Italy), were evaluated for inclusion into the study as of April 2011.

Inclusion criteria were age ≤30 years and serum 25(OH)D concentration <30 ng/mL.[29] Exclusion criteria were hyperparathyroidism, as detected by an intact serum parathyroid hormone (PTH) ≥65 pg/mL; Black ethnic group; supplementation with vitamin D in the previous 12 months; use of any treatment known to alter vitamin D status in the previous 6 months (excluding ARV); and any concomitant severe illness.

Persons of African descent were excluded to avoid possible confounding due to racial differences in vitamin D metabolism status, for which genetic explanations beyond possible skin color gradations have been identified.[32]

Patients with 25(OH)D <30 ng/mL with concomitant PTH level above normal limits were not considered eligible for the present study and were referred to treatment.

Study Design

A repeated-measures parallel-group RCT was performed. Eligible subjects were allocated to receive oral cholecalciferol or placebo using a 4-block randomized design.[33]

Serum 25(OH)D, 1,25$(OH)_2$D, PTH, calcium, urinary calcium, and CD4+ T cells were assessed 3 months before enrollment (–3 months), at baseline (0 months), and at each visit thereafter (3, 6, 9, and 12 months). T-lymphocyte VDR expression and Th1-, Th2-, Th17-, and Treg-lymphocytes were measured at 0, 3, and 12 months. Adverse events were defined as those injuries related to or caused by the treatments under study. Patients and/or legal guardians were specifically asked about adverse events at each study visit.

The main outcome was the frequency of hypovitaminosis D at the end of the trial, 3 months after the last dose. Twenty-five subjects per group gave a power of 83% to detect a reduction of 40% in the frequency of hypovitaminosis D in the vitamin D versus the placebo group at an alpha level of 0.05 (Fisher exact test).

The study was approved by the Luigi Sacco Hospital Ethical Committee and registered as trial number 2011–00059354 in the Eudract Registry. Written informed consent was obtained from the subjects or their legal guardians if they were younger than 18 years.

Intervention

Patients were randomized to receive orally vitamin D3 100,000 IU (Dibase, ABIOGEN PHARMA SpA) or matching placebo oil suspended in 2 mL of olive oil in sealed plastic syringes labeled with the unique identification numbers. All the study participants, outcome assessors (laboratory technicians and immunologists), and personnel, except

the pediatrician who administered the treatment, were blinded to it.

Direct observed treatment (DOT) was administered at the study site at baseline and at months 3, 6, and 9 for a cumulative dose of 400,000 IU.

Blood Collection

Blood was collected by venipuncture using EDTA-containing Vacutainer tubes (Becton Dickinson, Italy), and peripheral blood mononuclear cells (PBMC) were separated on lymphocyte separation medium (Organon Teknica, USA). Serum was obtained by centrifugation, and aliquot samples were stored at –80°C pending further analysis.

Vitamin D and Related Measures

Serum 25(OH)D was measured by immunoassay (25-hydroxy-vitamin D EIA; Immunodiagnostic Systems Ltd, Boldon, UK). The sensitivity of the assay was 5 nmol/L, with intra-assay precision ranging from 5.3% to 6.7% and inter-assay precision from 4.6% to 8.7%.

Serum $1,25(OH)_2D$ was measured by radioimmunoassay (1,25-dihydroxy vitamin D RIA; Immunodiagnostic Systems Ltd). The sensitivity of the assay was 5 pmol/L, with intra-assay precision ranging from 11.9% to 20.0% and inter-assay precision from 8.6% to 16.6%. Serum PTH was measured by immunoassay (Elecsys PTH Test System, Roche Diagnostics GmbH, Mannheim, Germany). The sensitivity of the assay was 1.2 pg/mL; intra-assay precision ranged from 2.6% to 6.5% and inter-assay precision from 1.1% to 4.1%. Serum and urinary calcium (spot sampling) were measured using standard laboratory techniques.

T-Cell Immunophenotyping

PBMCs were incubated for 18 hours in the presence/absence of a pool of gag+env peptides (HIV). During the last 6 hours, 10 μg/mL of brefeldin A (Sigma-Aldrich, St. Louis, MO, USA) was added to cell cultures for cytokine analyses.

We measured VDR expression on CD4+ T cells as previously described.[34]

Briefly, 0.5 x 10^6 PBMC were washed in phosphate-buffered saline (PBS) and stained for CD4 FITC monoclonal antibodies (Beckman-Coulter, Fullerton, CA, USA) for 15 minutes at room temperature in the dark. Cells were fixed in 1% paraformaldehyde (Sigma-Aldrich, St. Louis, MO, USA) for 15 minutes at 4°C, washed, resuspended in 0.5% saponin (Sigma-Aldrich), stained for VDR antibodies (R&D Systems Inc., Minneapolis, MN, USA), and coupled to phycoerythrin (PE) (Innova Biosciences, Cambridge, UK). After a 45-minute incubation at 4°C in the dark, cells were washed and fixed in 1% paraformaldehyde. The following antibody panels were used to define Th1, Th2, Th17, and Treg cells: anti-CD4 PE-Cy7, anti-IL2 PE, anti-IFN-γ FITC (Beckman-Coulter, Fullerton, CA, USA), anti-Tbet PE-Cy5 (eBioscience, San Diego, CA, USA), anti-CD4 PE-Cy7 (Beckman-Coulter), anti-GATA3 eFluor660 (eBioscience), anti-IL-4 PE (R&D Systems Inc), anti-CD4 PE-Cy5 (Beckman-Coulter), anti-ROR-gamma PE, anti-IL17A FITC (eBioscience), anti-CD4 PE-Cy7, anti-CD25 PE (Beckman-Coulter), anti-Foxp3 PE-Cy5 (eBioscience), and anti-IL10 FITC (R&D Systems Inc.).

Stimulated PBMCs were washed in PBS (Euroclone, Pero, Italy) and stained with surface antibodies. PBMCs were then washed, fixed, permeabilized (FoxP3 Staining Buffer Set; eBioscience), and stained with intracellular antibodies. Cells were finally washed with permeabilization buffer and acquired on an FC500 flow cytometer (Beckman Coulter, Brea, CA, USA). Th1 cells were identified as IFNγ- or/and IL2-secreting Tbet+ CD4+ T cells, Th2 cells were identified as IL4- or/and IL10-secreting GATA3+ CD4+ T cells, and Th17 cells were identified as IL17-secreting RORγt+ CD4+ T cells.

Data were analyzed by first gating on the lymphocyte population as defined by forward and side light scatters. From this population, a single color CD4 or CD8 histogram was made, and T cells were selected and inserted into a 2-dimensional dot plot.

Statistical Analysis

Most variables were not normally distributed, and baseline values are reported as 50th, 25th, and 75th percentiles. Categorical variables are reported as the number of subjects with the characteristic of interest. Between-group comparisons of categorical variables were performed using Fisher exact test. The longitudinal changes of the outcomes of interest were evaluated using mixed

linear regression models employing (1) treatment (categorical: 0 = placebo, 1 = vitamin D); (2) time [categorical: –3, 0, 3, 6, 9, and 12 months for 25(OH)D, $1,25(OH)_2D$, PTH, serum calcium, urinary calcium, and CD4+ T-cells and 0, 3 and 12 months for square-root transformed VDR, Th1-lymphocytes, Th2-lymphocytes, Th1:Th2 ratio, Th17-lymphocytes, Treg-lymphocytes, and Th17:Treg ratio], (3) a Treatment x Time interaction (Categorical x Categorical), and (4) the baseline value of the outcome as covariates and the patient as random effect.[35] Standardized residuals and level-1 residuals were evaluated to check model fit.[36]Although model fit improved substantially after square-root transformation of VDR, Th1-lymphocytes, Th2-lymphocytes, Th1:Th2 ratio, Th17-lymphocytes, Treg-lymphocytes, and Th17:Treg ratio, we nonetheless used robust 95% confidence intervals to relax the assumption of homoscedasticity of level-1 residuals. Although not strictly needed for the other outcomes, we calculated robust 95% confidence intervals for all models for reasons of uniformity. Using maximum likelihood estimation, the mixed models allowed us to manage missing data under the assumption of missing at random (MAR).[37] The analysis was intention-to-treat (ITT) for all outcomes. Statistical analysis was performed using Stata 12.1 (Stata Corp, College Station, TX, USA).

RESULTS

The selection of patients is depicted in **Figure 1**. Out of 90 vertically HIV-infected patients aged 8 to 26 years who underwent vitamin D status assessment 3 months before starting the trial (April 2011), 79 (87.7%) had vitamin D insufficiency [25(OH)D < 30 ng/mL]. Eleven subjects were excluded because of Black ethnicity; 11 patients with hypovitaminosis D and hyperparathyroidism were not eligible for randomization and were referred to treatment.

Of the remaining 57 patients, 52 agreed to participate in the trial and were randomized into the vitamin D (n = 26) or placebo group (n = 26). One patient in the vitamin D group developed epilepsy and 1 in the placebo group moved away from Milan before starting the study, so 50 patients were actually evaluated.

Baseline patients' characteristics are given in **Tables 1** and **2**. At enrollment, the 2 groups were comparable in both categorical (**Table 1**) and continuous measurements (**Table 2**). Twenty-one subjects (84%) in the vitamin D group and 22 patients (88%) receiving placebo had been under stable ARV therapy for at least 6 months before enrollment and all of them had undetectable HIV RNA levels (**Table 1**). The majority (52%) of the patients were receiving an NNRTI-based regimen; 17 (34%) subjects were receiving a protease inhibitor–based regimen. Only a few patients were naïve to ARV (**Table 1**).

Vitamin D and related measures as well as immunological features were also comparable in the 2 groups (**Table 2**).

Forty-four patients performed all scheduled visits. Given the DOT strategy, we reported no missed doses of vitamin D. Four patients in the vitamin D group missed 1 visit, while 2 in the placebo group were lost at follow-up at 3 and 6 months. Only 65% of the planned urinary calcium measurements were performed, whereas all other measurements were obtained in virtually all cases (from 98% to 100%). No patients were excluded from analysis at any time point, and the analysis was ITT.

Vitamin D and Related Outcomes

Changes in vitamin D and related outcomes during the study are reported in **Table 3**.

There were no changes in 25(OH)D, $1,25(OH)_2D$, and PTH levels at 0 (baseline) versus -3 months. At the first assessment after baseline (3 months), 25(OH)D levels were higher and PTH lower in the vitamin D group, but no between-group difference in $1,25(OH)_2D$ levels was detected. At the second assessment (6 months), an increase in $1,25(OH)_2D$ concentration was evident in the vitamin D group in addition to the increase in 25(OH)D and decrease in PTH. At the third (9 months) and fourth (12 months) assessments, these changes were still evident and stable.

Serum calcium showed a tendency to increase in the vitamin D group at 3 months, but the effect size was very modest and not replicated at the following time points. No changes in urinary calcium levels were seen in the 2 groups at any time. However, the changes in urinary calcium concentration need to be interpreted with caution because of the high number of missing data (35%).

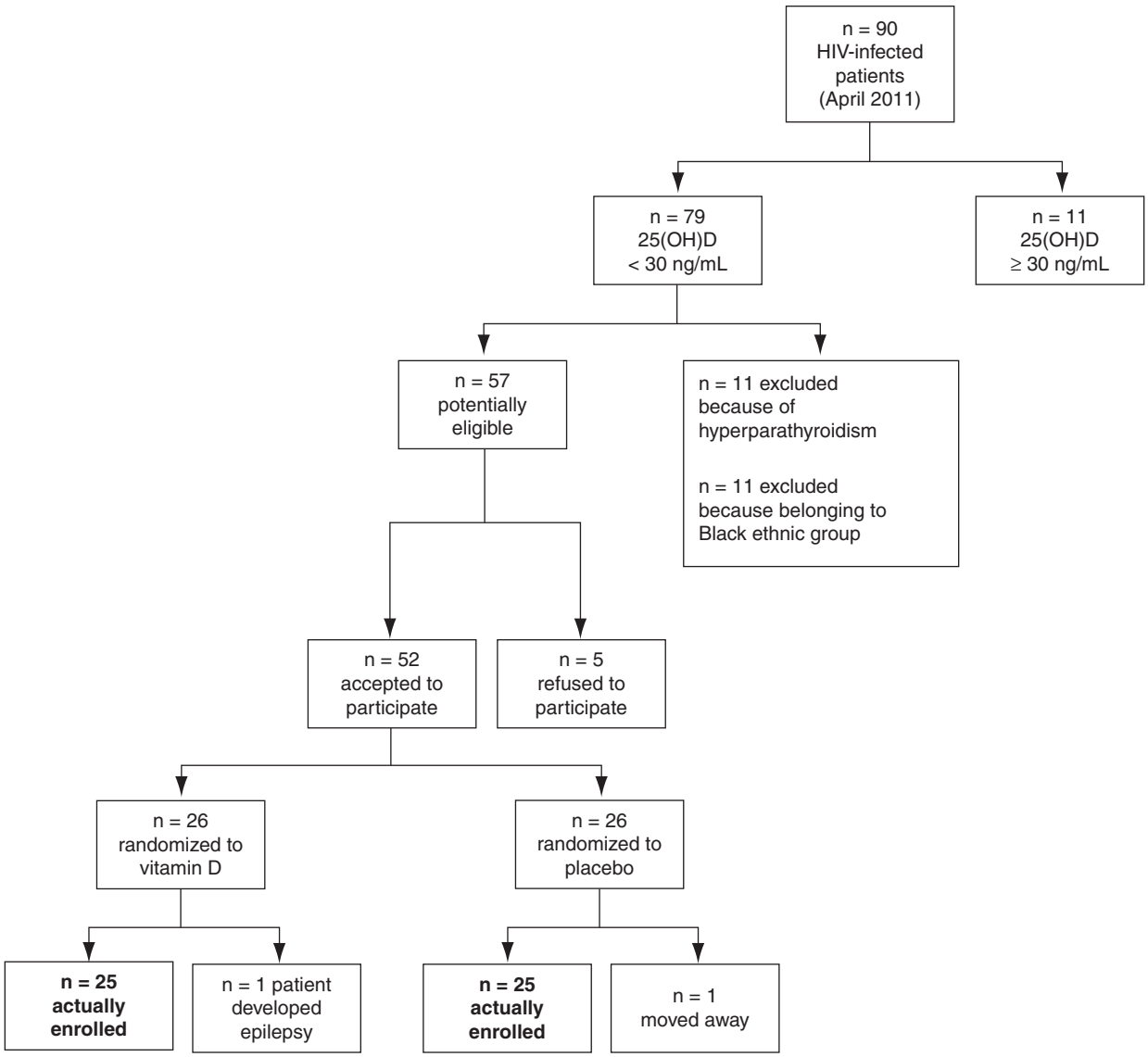

Figure 1. Selection of patients.

Figure 2 plots 25(OH)D values in the 2 groups during the study period. (These values were predicted from the mixed regression model of **Table 3** and take into account missing data.)

The frequency of vitamin D insufficiency at 12 months (main outcome) was 60% in the placebo group and 20% in the vitamin D group (P = .007). Data were calculated on 48 patients, because 2 patients in the placebo group were lost to follow-up during the study (see above).

Immunological Outcomes

No changes were observed in CD4+ T-cell count or percentage in the 2 groups (**Table 3**). A decrease of the Th17:Treg ratio (square-root transformed) was detected at 3 months in the vitamin D versus the placebo group, but it became nonsignificant at 12 months (**Table 4**).

Safety

No adverse events were reported in either group.

Table 1. Baseline categorical measurements of the participants

Category	Vitamin D (n = 25)	Placebo (n = 25)
CDC clinical category		
N+A	16	9
B+C	9	16
Current CDC clinical category		
N+A	25	25
B+C	0	0
CDC immune category		
1	8	5
2	10	10
3	7	10
Current CDC immune category		
1	19	21
2	6	4
3	0	0
Undetectable HIV-RNA (< 37 copies mL)	18	21
Treated with NNRTIs	13	13
Treated with PIs	8	9
Naïve	4	3

Note: CDC = Centers for Disease Control and Prevention; NNRTIs = non-nucleoside reverse transcriptase inhibitors; PIs = protease inhibitors.

Table 2. Baseline continuous measurements of the participants

	Vitamin D (n = 25)	Placebo (n = 25)
	P_{50th} (P_{25}–P_{75th})	P_{50th} (P_{25}–P_{75th})
Age, years	20 (14–23)	18 (15–23)
25(OH)D, ng/mL	15 (12–19)	15 (14–19)
$1,25(OH)_2D$, pg/mL	97 (86–113)	93 (70–108)
PTH, pg/mL	40 (25–48)	37 (32–41)
Serum calcium, mg/dL	9 (9–10)	10 (9–10)
Urinary calcium, mg/dL	11 (7–21)	12 (7–30)
CD4+ T cells, cells/mm^3	663 (507–796)	673 (601–773)
CD4+ T cells, %	37 (27–40)	34 (25–40)
Th1 lymphocytes, %	0.1 (0.1–0.4)	0.1 (0.0–0.2)
Th2 lymphocytes, %	0.5 (0.2–1.1)	0.7 (0.3–1.1)
Th1:Th2 ratio	0.2 (0.1–0.7)	0.2 (0.0–0.4)
Th17 lymphocytes, %	0.3 (0.1–0.3)	0.2 (0.0–0.3)
Treg lymphocytes, %	0.3 (0.2–0.4)	0.4 (0.2–0.7)
Th17:Treg ratio	1.0 (0.4–1.5)	0.2 (0.0–0.7)
Lymphocyte VDR, %	6.9 (2.4–11.9)	8.6 (4.2–13.7)

Note: Values given as % (range) or n (range). P_x = xth percentile (P_{50} is the median); PTH = parathyroid hormone; VDR = vitamin D receptor.

Table 3. Changes of vitamin D and related outcomes for vitamin D vs placebo group

	Change vs –3 months for vitamin D vs placebo						
Month	25-OH-D, ng/mL	$1,25(OH)_2D$, pg/mL	PTH, pg/mL	Serum calcium, mg/dL	Urinary calcium, mg/dL	CD4+ T cells, cells/mm^3	CD4+ T cells, %
0	0.0 [–3.0,3.0]	13.0 [–4.4,30.4]	–3.6 [–10.9,3.7]	–0.1 [–0.3,0.1]	–1.2 [–6.1,3.6]	–72.4 [–186.8,42.1]	–1.4 [–4.6,1.8]
3	4.8* [1.5,8.1]	13.6 [–3.3,30.5]	–10.3** [–17.6,–3.0]	0.2* [0.0,0.4]	–5.7 [–19.3,8.0]	7.0 [–87.1,101.2]	0.9 [–2.1,3.9]
6	11.1** [3.8,18.3]	27.9** [10.1,45.8]	–12.4** [–20.3,–4.5]	0.1 [–0.2,0.3]	2.5 [–6.2,11.1]	19.9 [–119.6,159.3]	–2.7 [–8.3,2.8]
9	8.9*** [5.9,11.9]	23.9* [2.7,45.1]	–13.3** [–23.2,–3.4]	4.0 [–3.8,11.9]	5.6 [–1.6,12.8]	48.6 [–68.9,166.0]	–1.2 [–5.1,2.6]
12	12.5*** [5.9,19.0]	27.0** [10.0,44.0]	–9.8* [–18.2,–1.5]	0.3 [–0.2,0.9]	7.4 [–0.8,15.5]	58.1 [–114.5,230.7]	–77.0 [–180.2,26.3]

Note: 95% robust confidence intervals in brackets. Point estimates and confidence intervals were obtained from a mixed linear model.
*$P < .05$. **$P < .01$. ***$P < .001$.

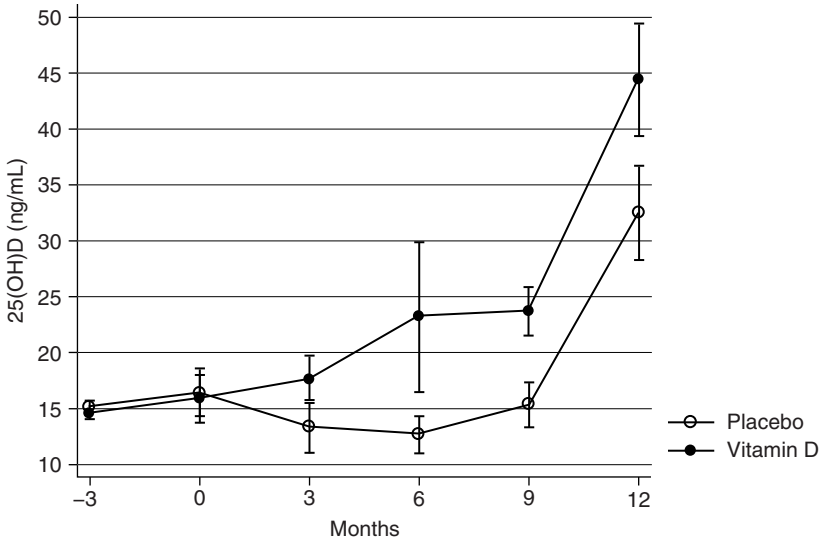

Figure 2. 25(OH)D levels during the study: point estimates and confidence intervals obtained from a missed linear model.

Table 4. Changes in immunological outcomes for vitamin D vs placebo group

	Change vs 0 months for vitamin D vs placebo						
	VDR[a]	Th1[a]	Th2[a]	Th1:Th2[a]	Th17[a]	Treg[a]	Th17:Treg[a]
Month 3	0.6 [–0.2, 1.4]	–0.3 [–0.6, 0.1]	–0.1 [–0.4, 0.2]	–0.5 [–1.0, 0.1]	–0.2 [–0.5, 0.0]	0.1 [–0.2, 0.4]	–0.6 [–1.0,–0.1]
Month 12	0.5 [–0.2, 1.3]	–0.1 [–0.7, 0.5]	0.3 [–0.1, 0.6]	–0.7 [–2.2, 0.8]	0.1 [–0.2, 0.5]	0.7 [–0.2, 1.5]	–0.4 [–0.8, 0.1]

Note: 95% robust confidence intervals in brackets. Point estimates and confidence intervals were obtained from a mixed linear model.

[a]After square-root transformation.

DISCUSSION

This is the first RCT aimed to evaluate changes in 25(OH)D, $1,25(OH)_2D$, and PTH levels in HIV-infected youth undergoing vitamin D3 supplementation over a long period (12 months) and, concomitantly, to test whether such supplementation may affect the T-cell phenotype.

Given the risk of poor adherence to vitamin supplementation among adolescents, we chose an every-3-months schedule, which allowed us to perform the direct observed therapy concomitantly to the scheduled follow-up visits in our clinic.

First, our study shows that cholecalciferol supplementation increased 25(OH)D and $1,25(OH)_2D$ and decreased PTH levels in HIV-infected youth with a relatively preserved immunological function. 25(OH)D increased and PTH decreased 3 months after the first administration of cholecalciferol, whereas the increase in $1,25(OH)_2D$ took 6 months to become apparent. Thus, PTH appeared to be an early marker of vitamin D status. This is important in view of the fact that current guidelines consider the effect of cholecalciferol on PTH to be an important criterion to determine whether vitamin D supplementation is needed.[29] At 12 months, the

mean (95% CI) difference in vitamin D was 27 (10 to 44) ng/mL for the vitamin D group versus the placebo group ($P < .001$). At the same time, the frequency of vitamin D insufficiency [ie, 25(OH)D < 30 ng/mL] was 60% in the placebo group and 20% in the vitamin D group ($P = .007$). It is important to note the increase in 25(OH)D that occurred in both study groups between months 6 and 12 (**Figure 1**). A possible explanation for this finding is that the last 3 months of our trial took place during summer. It is well known that circulating levels of vitamin D tend to increase during summer,[29] and this has been reported also in HIV-infected children.[5] In addition to the possible effect of sunlight exposure on vitamin D status, our findings agree with those of Arpadi and colleagues,[23] who showed that a bimonthly supplementation with cholecalciferol (100,000 IU) and calcium (1 g/day) was effective in improving vitamin D status in HIV-infected children.

1α-Hydroxylation of 25(OH)D to $1,25(OH)_2D$ may be impaired in HIV-infected subjects. In our patients supplemented with vitamin D, both serum 25(OH)D and $1,25(OH)_2D$ increased, although the latter effect took more time to be evident. This finding may be partly explained by the fact that our patients had a relatively preserved immune function.[30]

Second, although vitamin D had no effect on CD4+ T cells, it is of some interest that in our cohort cholecalciferol supplementation was associated with a decrease in the Th17:Treg ratio, which appears to be the principal novelty of the present investigation.

On the basis of observational data, it has been hypothesized that serum 1,25 vitamin D concentration correlates with degree of immunodeficiency[38,39] or CD4 recovery in HIV-infected adult males.[40] Our finding of no association between vitamin D supplementation and CD4+ T cells is in keeping with the results of Kakalia et al,[41] who showed that in HIV-infected adults with relatively preserved immune function, vitamin D supplementation does not lead to changes of CD4+ T cells. Data obtained mostly from in vitro and animal studies[26,42] show that, with respect to the adaptive immune system, $1,25(OH)_2D$ stimulates a Th2-cytokine profile and the development of Treg lymphocytes with possible inhibition of Th1- and Th17-subsets. Our study is the first to examine markers of adaptive immunity in an RCT involving vitamin D supplementation in HIV-infected children and young adults. Although this was a secondary outcome for which no formal sample size calculation was performed, it is interesting to see that the observed changes point in the same direction as available studies. We found in fact a decreased Th17:Treg ratio at 3 months in the vitamin D group compared to the placebo group. Although the changes of the numerator and denominator of this ratio were not significant, the ratio clearly changed because of a decrease in Th17- and a concomitant increase in Treg lymphocytes.

Treg lymphocytes play a pivotal role in immune activation, and their function in HIV infection is attracting much interest. These cells suppress HIV-specific adaptive immune responses,[43] and they may control excessive immune activation.[44] In a recent study in HIV-infected adults, an increase in $1,25(OH)_2D$ was accompanied by an expansion of Treg lymphocytes.[45] Vitamin D up-regulates Th2-dependent cytokine expression and lowers Th1-dependent cytokine levels.[46] In the course of HIV infection, a shift from Th1- to Th2-lymphocytes is of concern, because it may be associated with disease progression and greater immunosuppression.[47,48] In our trial, we found no evidence of potentially relevant changes in the Th1:Th2 ratio.

Our study has some limitations. First, our patients had a relatively preserved immune function, and our findings cannot be generalized to subjects with more advanced HIV disease. Second, we studied Caucasian youths living in the Milan metropolitan area, and our findings are unlikely to apply to subjects of different ethnicities or those living at different latitudes. Third, we did not evaluate the changes of vitamin D intake with foods during the study. Fourth, calcium:creatinine ratios are not displayed because only 65% of the planned urinary calcium measurements were obtained. Last, we are aware of the fact that our study is underpowered to determine whether the chosen supplementation may affect CD4+ T-cell counts.

CONCLUSION

In our cohort of HIV-infected children and young adults, a 12-month oral supplementation with 100,000 IU of cholecalciferol given every 3 months produced an early decrease in PTH, a concomitant increase in 25(OH)D, and a later increase in $1,25(OH)_2D$ levels. Such treatment had no effect on CD4+ T cells but was associated with a decreased Th17:Treg ratio at 3 months.

Vitamin D might thus produce potentially important changes in the T-cell phenotype warranting further studies in larger cohorts.

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