Prevalence and risk factors for efavirenz-based antiretroviral treatment–associated severe vitamin D deficiency
A prospective cohort study
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Abstract
Initiation of efavirenz-based combination antiretroviral therapy (cART) is associated with Vitamin D deficiency, but the risk factors including efavirenz pharmacokinetics for cART-induced severe vitamin D deficiency (SVDD) and the impact of anti-tuberculosis (TB) co-treatment are not explored. We investigated the prevalence of SVDD in HIV and TB-HIV coinfected patients and associated risk factors for treatment-induced SVDD.

Treatment-naïve Ethiopian HIV patients with (n = 102) or without (n = 89) TB co-infection were enrolled prospectively and received efavirenz-based cART. In TB-HIV coinfected patients, rifampicin-based anti-TB treatment was initiated 4 or 8 weeks before starting cART. Plasma 25-hydroxyvitamin D (25 [OH]D), cholesterol and 4-beta hydroxycholesterol concentrations were measured at baseline, 4th, 16th, and 48th week of cART. Plasma efavirenz concentrations were determined at 4th and 16th weeks of cART.

TB-HIV patients had significantly lower plasma 25 (OH)D3 levels than HIV-only patients at baseline. TB co-infection, low Karnofsky score, high viral load, and high CYP3A activity as measured by plasma 4β-hydroxycholesterol/cholesterol ratios were significant predictors of low 25 (OH)D3 levels at baseline. In HIV-only patients, initiation of efavirenz-based cART increased the prevalence of SVDD from 27% at baseline to 76%, 79%, and 43% at 4th, 16th, and 48th weeks of cART, respectively. The median 25 (OH)D3 levels declined from baseline by −40%, −50%, and −14% at 4th, 16th, and 48th weeks of cART, respectively.

In TB-HIV patients, previous anti-TB therapy had no influence on 25 (OH)D3 levels, but the initiation of efavirenz-based cART increased the prevalence of SVDD from 57% at baseline to 70% and 72% at the 4th and 16th weeks of cART, respectively. Median plasma 25 (OH)D3 declined from baseline by −17% and −21% at week 4 and 16 of cART, respectively.

Our results indicate low plasma cholesterol, high CYP3A activity, and high plasma efavirenz concentrations as significant predictors of early efavirenz-based cART-induced vitamin D deficiency. Low plasma 25 (OH)D3 level at baseline is associated with TB co-infection and HIV diseases progression. Initiation of efavirenz-based cART is associated with high incidence of SVDD, whereas rifampicin based anti-TB therapy co-treatment has no significant effect. Supplementary vitamin D during cART initiation may be beneficial for HIV patients regardless of TB coinfection.

Abbreviations: cART = combination antiretroviral therapy, CYP = cytochrome P450, HIV = human immunodeficiency virus, SVDD = severe vitamin D deficiency, TB = tuberculosis, VDR = vitamin D receptor.

Keywords: 25-hydroxyvitamin D, cART, CYP2B6, CYP3A4, cytochrome P450, drug metabolism, HIV, TB, tuberculosis, VDR, vitamin D receptor, vitamin D
1. Introduction

Vitamin D deficiency is associated with many chronic illnesses, including autoimmune, cardiovascular, and infectious diseases like tuberculosis (TB), HIV disease progression, and mortality.\(^\text{[1]-[3]}\) Plasma 25 (OH)\(_2\)D, the primary circulating form of vitamin D, serves as an indicator of vitamin D status.\(^\text{[4]}\) Vitamin D3 is formed naturally from 7-dehydrocholesterol, a cholesterol precursor, in the skin upon exposure to sunlight. Thus factors influencing 7-dehydrocholesterol levels may result in altered vitamin D and cholesterol levels. Efavirenz-based combination antiretroviral therapy (cART) is associated with changes in both vitamin D\(^\text{[5]-[7]}\) and cholesterol concentrations,\(^\text{[10]}\) but it is unclear whether there is a correlation between the variation in vitamin D and cholesterol levels during cART and whether this effect is related to drug concentrations.

TB is the most common opportunistic infection and the leading cause of death in people living with HIV. Concomitant HIV and TB treatments are challenging because significant drug–drug interactions, overlapping toxicities, and immune reconstitution inflammatory syndrome.\(^\text{[11]-[13]}\) Efavirenz and rifampicin, the cornerstones of first-line cART and anti-TB treatments, respectively, are potent inducers CYP2B6 and CYP3A enzymes and transporter proteins.\(^\text{[10,14,15]}\) Efavirenz is mainly metabolized by genetically polymorphic CYP2B6 and to some extent by CYP3A enzymes. Vitamin D is involved in the regulation of CYP3A and CYP2B6 enzyme expression.\(^\text{[16,17]}\) However, CYP3A catalyzes the 4-hydroxylation of 25 (OH)\(_2\)D, and hence CYP3A induction may contribute to drug-induced vitamin D deficiency.\(^\text{[16,18]}\) Long-term CYP2B6 and CYP3A induction by efavirenz is influenced by pharmacogenetic factors relevant for efavirenz disposition.\(^\text{[10,15,20-22]}\) Accordingly, host genetic factors affecting the plasma concentration of CYP3A inducers may also potentially affect the vitamin D levels in patients on long-term treatment with efavirenz or rifampicin. Indeed, a CYP2B6 genotype-based efavirenz dose adjustment was recommended recently to optimize treatment outcomes.\(^\text{[23,24]}\) Implication of inter-individual variations in efavirenz plasma concentration for efavirenz-induced vitamin D deficiency is yet to be investigated.

Previous studies reported that efavirenz-based cART lowers the 25 (OH)\(_2\)D levels.\(^\text{[15-9]}\) Poor absorption, lower exposure to the sunshine, and darker skin pigmentation are known risk factors for low 25 (OH)\(_2\)D levels.\(^\text{[25]}\) Data regarding predictors of 25 (OH)\(_2\)D levels during cART are scarce in HIV patients living in tropical areas, where there is an all-year round sunshine. The risk factors for cART-induced severe vitamin D deficiency and the implication of anti-TB co-treatment remain to be investigated, especially in sub-Saharan Africa, where both HIV and TB are major public health problems. In the present study, we hypothesized that high CYP3A induction by rifampicin co-treatment and inter-individual variability in efavirenz disposition may play a role for between-patient variability in vitamin D status during cART. Therefore, the objectives of this study were to identify the prevalence and associated risk factors for low 25 (OH)\(_2\)D levels and severe vitamin D deficiency before starting treatment and during efavirenz-based cART alone or together with rifampicin-based anti-TB co-therapy in HIV or TB-HIV co-infected patients, respectively.

2. Materials and methods

2.1. Study design and settings

The study design was prospective, comparative, observational, open-label, parallel assignment, 2-arm pharmacogenetic and pharmacokinetic cohort study to identify the prevalence and risk factors for severe vitamin D deficiency at baseline and during efavirenz-based cART with or without rifampicin-based anti-TB therapy. The study was conducted between June 2008 and June 2011 at HIV and TB clinics in Addis Ababa (latitude 9-1° N, Ethiopia).

All patients gave written, informed consent to participate in this study. The study protocol received ethics approvals from the Institutional Review Board (IRB) of School of Medicine, Addis Ababa University, National Ethics Review Committee of Ethiopia. The study also received approval from IRB of Karolinska Institutet (Stockholm, Sweden) and was conducted as per International Conference for Harmonization-Good Clinical Practice (ICH-GCP) guidelines.

2.2. Study participants

This study was conducted as one of the substudies designed under the umbrella of the broad clinical research project entitled “The HIV-TB Pharmagenic Study” in Ethiopia. Details of the main study design, patient enrolment process, and inclusion criteria with follow-up and drug treatments were reported previously.\(^\text{[26]}\) Briefly, for the main study, newly diagnosed HIV-infected (n = 285) and TB-HIV co-infected (n = 208) patients were recruited prospectively and enrolled in parallel and followed up to 48 weeks. The eligibility criteria were ≥18 years of age, not pregnant, and CD4 count ≤200 cells/mm\(^3\). None of the study participants received isoniazid prophylaxis or other TB treatment for 2 years before enrollment. Treatment adherence was assessed by self-report.

For the present study, a total of 191 patients (102 TB-HIV co-infected patients and 89 HIV only infected patients), with complete set of plasma samples collected at baseline, and at the 4th and 16th weeks of cART, were used to monitor the change in vitamin D during cART. The sample size was calculated for each treatment group considering a moderate effect size, \(\text{E} = 0.3\), to detect the change in vitamin D levels before and after efavirenz exposure (paired \(t\) test) with 80% of study power and \(\text{n} = 5\%\), the desired sample size was calculated as 87 in each group. Plasma sample collected at 48 weeks of cART from 42 HIV-only patients was also available and used.

2.3. Treatment

All HIV patients received cART (600 mg efavirenz-based cART containing either zidovudine/lamivudine/efavirenz or stavudine/ lamivudine/efavirenz). Plasma samples for the determination of plasma cholesterol, 4β-hydroxycholesterol, and 25 (OH)\(_2\)D were taken before the initiation of treatment (week 0) and at weeks 4, 16, and 48 of treatment.

All TB-HIV patients (n = 102) initiated rifampicin-based anti-TB treatment 4 (n = 69) or 8 weeks (n = 33) before the initiation of efavirenz-based cART. The short course anti-TB treatment consisted of an initiation phase with rifampicin/isoniazid/pyrazinamide /ethambutol for 2 months followed by a continuation phase with rifampicin/isoniazid for 4 months. Samples for the determination of plasma cholesterol, 4β-hydroxycholesterol and 25 (OH)\(_2\)D concentrations were collected before starting anti-TB treatment (corresponding to week -4 or -8 weeks of cART), at the initiation of cART (week 0) and weeks 4 and 16 of cART. The study population, follow-up period, and study sampling time points are presented in Figure 1.
2.5. Quantification of plasma cholesterol concentrations

Cholesterol was determined in plasma using a commercial enzymatic method (Cholesterol Chod-PAP, Roche Diagnostics GMBH, Mannheim, Germany) on a Roche/Hitachi Modular Instrument. The between-day variation was 1.3% at 5 mmol/L.

2.6. Quantification of efavirenz plasma concentrations

Plasma samples for determination of efavirenz were collected at weeks 4 and 16 of cART in both treatment groups. Plasma efavirenz concentrations were determined using LC-MS/MS as described previously.[20,26,29] Briefly, protein precipitation was performed using acetonitrile containing internal standards (\(\text{\textsuperscript{13}}\)C-efavirenz and \(\text{\textsuperscript{3}}\)H\(_4\)-8-hydroxy-efavirenz, respectively). Analysis was performed using a Synergy Fusion RP chromatography column (Phenomenex, Torrance, CA) and mobile phases containing ammonium acetate (5 mmol/L, acidic), methanol, and acetonitrile. The lower limits of quantification in plasma were 10.0 ng/mL. The efavirenz calibration range was 10 to 10000 ng/mL. The method was validated according to the FDA validation guidelines and fulfilled all criteria concerning accuracy, precision, recovery, linearity, and stability.

2.7. Statistical analysis

Comparison of median plasma \(\text{\textsuperscript{25}}\)OH\(_3\) levels between treatment groups was done using the Mann-Whitney test. Plasma \(\text{\textsuperscript{25}}\)OH\(_3\), cholesterol, efavirenz concentrations, and 4\(\beta\)-hydroxycholesterol/cholesterol ratios were log-transformed (base 10) before applying the test, repeated measure analysis of variance (ANOVA), and regression analysis. Pairwise comparison of data from baseline within and between treatment groups was made using paired and unpaired t test, respectively. For each patient, the percent change in plasma \(\text{\textsuperscript{25}}\)OH\(_3\) level from the 4\(^{th}\), 16\(^{th}\) and 48\(^{th}\) weeks of cART was calculated using the following formula:

\[
\% \text{ change in plasma } \text{\textsuperscript{25}}\text{OH}\text{D}_3 = \frac{25(\text{OH})D_3 \text{ at week } x - 25(\text{OH})D_3 \text{ at baseline}}{25(\text{OH})D_3 \text{ at baseline}} \times 100
\]

Repeated measure ANOVA was used to analyze the change in log plasma \(\text{\textsuperscript{25}}\)OH\(_3\) levels over time. Univariate followed by multivariate linear regression analysis was performed to identify predictors of low plasma \(\text{\textsuperscript{25}}\)OH\(_3\) levels at baseline and during treatment. Predictor variables that resulted in a P value <0.1 in the univariate regression analysis was entered into a backward stepwise multivariate regression analysis to identify significant predictors in the final model. Likewise logistic regression was done to determine predictors of severe vitamin D deficiency before and during CART. Statistical analyses were performed using Statistica version 12 (StatSoftInc, Tulsa, OK) and SPSS Statistics (IBM Corporation, Somers, NY) software, version 23.0. GraphPad Prism version 5.0 for Windows (Graph Pad, La Jolla, CA) was used for graphical presentations. A P value <0.05 was considered significant.

3. Results

The baseline sociodemographic, clinical, and laboratory characteristics of study participants stratified by treatment group are presented in Table 1. The best measure of vitamin D status is the...
16 weeks of ART – 4 weeks of ART

Previous RIF-only treatment

76 23.9 (15.2 - <60)

patients had 25 (OH)D2 levels above the lower LOQ; hence, all (OH)D3 levels during therapy between the 2 treatment groups are

time on treatment – baseline (pretreatment) 102 23.2 (14.9 - 60 kg).

Table 1

Baseline demographic, clinical, and laboratory characteristics of the study participants.

|                | HIV-only, n = 89 | TB-HIV, n = 102 |
|----------------|------------------|-----------------|
| Sex            |                  |                 |
| Male           | 20 (22.5%)       | 47 (46.1%)      |
| Female         | 69 (77.5%)       | 55 (53.9%)      |
| HIV stage      |                  |                 |
| 1              | 1 (1.2%)         |                 |
| 2              | 7 (8.1%)         | 1 (1.0%)        |
| 3              | 38 (44.2%)       | 56 (56.6%)      |
| 4              | 40 (46.5%)       | 42 (42.4%)      |
| Type of ART    |                  |                 |
| d4T30/3TC/EFV  | 47 (54.7%)       | 38 (38.4%)      |
| d4T40/3TC/EFV  | 5 (5.8%)         | 1 (1.0%)        |
| TDF3/3TC/EFV   |                 | 26 (26.3%)      |
| ZDV3/3TC/EFV   | 34 (39.5%)       | 34 (34.3%)      |

Table 2

Comparison of median and IQR of plasma 25 (OH)D3 (nmol/L) levels at baseline and during treatment.

| Time on treatment | N  | Median (IQR) | Median % change (IQR) | HIV-only cohort | N  | Median (IQR) | Median % change (IQR) |
|-------------------|----|--------------|------------------------|-----------------|----|--------------|------------------------|
| Baseline (pretreatment) | 102 | 23.2 (14.9-33.2) | - | 89 | 32.5 (24.0-41.86) | - |
| Previous RIF-only treatment | 76 | 23.9 (15.2-35.4) | -1% (-17 to 48) | 88 | 19.95 (14.5-25.0) | -40% (-55 to -15) |
| 4 weeks of AR *  | 102 | 19.25 (12.2-28.0) | -17% (-37 to 16) | 88 | 19.95 (14.5-25.0) | -40% (-55 to -15) |
| 16 weeks of AR * | 94  | 16.85 (11.6-27.7) | -21% (-50 to 14) | 85 | 15.20 (10.5-22.3) | -50% (-63 to -31) |
| 48 Weeks of AR   | 40  | 20.29 (19.2-35.4) | -14% (-50 to 23) | 88 | 19.95 (14.5-25.0) | -40% (-55 to -15) |

* ART = antiretroviral therapy. RIF = rifampicin. IQR = interquartile range.

3TC = lamivudine, EFV = efavirenz, TDF = tenofovir, ZDV = zidovudine, d4T30 (stavudine 30 mg for patients weighing <60 kg), d4T40 (stavudine 40 mg for patients weighing ≥60 kg).

Median (interquartile range).

Concentration of 25 (OH)D3 + 25 (OH)D2 in plasma. Very few patients had 25 (OH)D2 levels above the lower LOQ; hence, all statistical calculations were done using only plasma 25 (OH)D3 data.

3.1. Plasma 25 (OH)D3 levels at baseline and during treatment

Comparisons of the median plasma 25 (OH)D3 levels at baseline and during cART and the median percent change in plasma 25 (OH)D3 levels during therapy between the 2 treatment groups are presented in Table 2. The pretreatment median plasma 25 (OH)D3 concentration was significantly lower in TB-HIV coinfected patients than in HIV patients without TB. In the HIV-only cohort, initiation of efavirenz-based cART dramatically lowered the median plasma 25 (OH)D3 levels by –40.0%, –50.0%, –14.0% at week 4, 16, and 48 of cART, respectively (Table 2). Although in TB-HIV co-infected patients, previous treatment with rifampicin-based anti-TB drugs for 4 or 8 weeks resulted in no significant change in plasma 25 (OH)D3 from baseline. However, initiation of efavirenz-based cART co-treatment lead to a gradual decline in plasma 25 (OH)D3 level and the median percent change of 25 (OH)D3 from baseline by 4th and 16th of weeks of cART was –17.0% and –21.0%, respectively.

Comparisons of change in log plasma 25 (OH)D3 levels from baseline after 4 weeks of efavirenz-based cART alone versus 4 or 8 weeks of rifampicin-based anti-TB therapy alone are presented in Figure 2. TB-HIV co-infected patients had significantly lower mean log plasma 25 (OH)D3 levels compared to HIV-only patients at baseline (P <0.001, Fig. 2), which became reversed soon after initiating therapy. The mean log plasma 25 (OH)D3 levels became significantly lower in HIV-only patients after 4 weeks of efavirenz-based cART compared to 4 to 8 weeks of rifampicin-based anti-TB treatment only in TB-HIV patients (P = 0.03). Initiation of cART in TB-HIV patients on anti-TB therapy lowered the mean plasma 25 (OH)D3 levels, and there was no significant difference in the mean plasma 25 (OH)D3 levels between the 2 treatment groups at weeks 4 and 16 of cART.

The overall change in the mean log plasma 25 (OH)D3 levels profile from baseline during efavirenz-based cART alone versus rifampicin-based anti-TB co-treatment is presented in Figure 3. In HIV-only patients, within-treatment group analysis over time (using repeated measure ANOVA) indicated that efavirenz-based cART significantly reduced the mean log plasma levels of 25 (OH)D3 (P <0.001). Paired t-tests indicated significantly lower plasma 25 (OH)D3 levels at week 4 (P <0.001, geometric mean ratio [GMR] = 1.606; 95% confidence interval [CI] of the mean GMR = 1.46-1.76) and at week 16 (P <0.001, GMR = 1.99; 95% CI of GMR = 1.76-2.21) compared with the baseline pretreatment value. As cART continued, the plasma 25 (OH)D3 levels were restored, and no significant difference from baseline was found after 48 weeks of cART (P =0.12) in HIV-only patients.

In TB-HIV co-infected patients, within-treatment group analysis indicated that efavirenz-based cART significantly reduced mean log plasma levels of 25 (OH)D3 (P <0.0001). Paired t-tests indicated significantly lower 25 (OH)D3 levels at week 4 (P =0.01, GMR = 1.17; 95% CI of GMR = 1.04-1.34) and week 16 (P <0.001, GMR = 1.41, 95% CI of GMR = 1.18-1.69) compared to the baseline value. Data on plasma 25 (OH)D3 at week 48 of cART in TB-HIV co-infected patients were not available.
3.2. Vitamin D deficiency at baseline and during treatment

All patients had deficient (92%) or insufficient (8%) plasma 25(OH)D3 levels at the study enrollment. Despite receiving cART and anti-TB therapy, none of the patients achieved a sufficient (>72.5 nmol/L) plasma 25(OH)D3 level during the study follow-up period.

The prevalence of SVDD before starting treatment (at baseline) and at the 4th, 16th, and 48th weeks of efavirenz-based cART in each treatment group is presented in Table 3. At baseline, 57% (95% CI = 48.3%–67.5%) of TB-HIV co-infected patients had SVDD, whereas only 27% (95% CI = 15.1%–32.9%) of HIV-
only infected patients had SVDD ($P < 0.001$). In the HIV-only cohort, initiation of efavirenz-based cART significantly increased the proportion of patients with SVDD from 27% at baseline to 76% (95% CI = 67.1%–84.5%) at week 4, and 79% (95% CI = 70.3%–87.3%) at week 16, and 42.5% (95% CI = 32.2%–52.7%) at week 48 cART. Whereas in TB-HIV patients on rifampicin-based anti-TB therapy, initiation of efavirenz-based cART co-treatment increased the proportion of patients with SVDD from 57% at baseline to 70% (95% CI = 69.7%–78.3%) at week 4 and 72% (95% CI = 63.6%–80.9%) at week 16 of cART.

### 3.3. Plasma cholesterol levels at baseline and during treatment

The pretreatment mean log plasma cholesterol level was significantly higher in HIV patients compared to TB-HIV patients. Initiation of cART in HIV-only patients significantly reduced the plasma level of cholesterol. In contrast, increased plasma levels of cholesterol after 4 to 8 weeks of rifampicin-based anti-TB therapy alone were observed in TB-HIV patients (Fig. 2). The median percent reduction from baseline in cholesterol level by 4 weeks of efavirenz-based cART was −33% and the respective increase by rifampicin-based anti-TB therapy treatment alone was 21%. There was a similar pattern of change in plasma cholesterol and 25 (OH)D₃ level particularly in HIV-only patients treated with efavirenz-based cART (Fig. 3).

### 3.4. Efavirenz plasma concentrations

The median (IQR) of efavirenz plasma concentrations in HIV-only and TB-HIV patients at week 4 of cART was 1200 (841–1846) ng/mL and 1574 (984–2041) ng/mL, respectively ($P = 0.45$). The median and IQR of efavirenz plasma concentrations in HIV-only and TB-HIV patients at week 16 of cART were 1328 (980–1947) ng/mL and 1236 (826–2013) ng/mL, respectively ($P = 0.26$).

### 3.5. Predictors of low vitamin D status at baseline and during treatment

Linear regression analysis was used to identify predictors of low plasma 25 (OH)D₃ at baseline (Table 4). In a univariate analysis, low plasma 25 (OH)D₃ levels at baseline were significantly associated with TB-co-infection, low Karnofsky score, low body mass index, low plasma cholesterol level, low hemoglobin level, low albumin, high viral load, high CYP3A activity (as measured by 4β-hydroxycholesterol to cholesterol ratio), and high serum alkaline phosphatase. In a stepwise multivariate regression analysis, low Karnofsky score, low albumin level, high viral load, and high 4β-hydroxycholesterol to cholesterol ratio remained significant predictors of low 25 (OH)D₃ concentration at baseline.

Univariate logistic regression identified TB co-infection, low Karnofsky score, high aspartate aminotransferase, low albumin levels, high 4β-hydroxycholesterol to cholesterol ratio, and high HIV viral load as significant predictors of SVDD at baseline. Multivariate logistic regression, using a backward stepwise conditional model of all variables with a $P$ value < 0.1 in the univariate analysis (Table 4), identified TB co-infection, high HIV viral load, and low albumin level as significant predictors of SVDD at baseline.

After 4 weeks of efavirenz-based cART, low Karnofsky score and albumin level at baseline, low plasma cholesterol, high efavirenz plasma concentration, and high 4β-hydroxycholesterol...
to cholesterol ratio at week 4 of cART were significant predictors of a low 25 (OH)D level soon after efavirenz-based cART initiation (Table 5). In a multivariate regression analysis, low Karnofsky score at baseline, high-current efavirenz plasma concentration, and high-current 4β-hydroxycholesterol to cholesterol ratio remained significant predictors of a low 25 (OH)D level at week 4 of cART (Table 5).

In a univariate logistic regression analysis, low albumin at baseline, low Karnofsky score at baseline, low cholesterol, and high-current 4β-hydroxycholesterol to cholesterol ratio were significant predictors of a low 25 (OH)D3 level soon after efavirenz-based cART (Table 5).

### 4. Discussion

We performed a prospective observational study to identify predictors of a low 25 (OH)D3 level before starting therapy and during efavirenz-based cART, anti-TB or a combination thereof in treatment-naive HIV patients with or without TB co-infection. The main finding of this study includes: a significant association of a low plasma 25 (OH)D3 level at baseline with TB co-infection and HIV disease progression; efavirenz-based cART significantly reduces plasma 25 (OH)D3 levels and this effect is more pronounced when given alone than with rifampicin-based anti-TB therapy; low plasma cholesterol level, high CYP3A activity, and high plasma efavirenz concentration are predictors of early cART-induced low 25 (OH)D3 level.

Seasonal variation affects vitamin D status, particularly in countries far from the equator, where the lower angle of the sun and more cloud cover during the winter result in less UV-B exposure and hence low production of vitamin D in skin.[30,31] However, lack of sunshine-derived vitamin D deficiency is not expected in countries around the equator.[32,33] Ethiopia is located in east Africa close to the equator (3 degree N to 14.8 degree latitude) and there is abundant sunshine to form vitamin D all year round.[34] This study was conducted in Addis Ababa, the capital city of Ethiopia, located at latitude 9 degree N. In the present study, we found no significant influence of seasonal variation (rainy versus dry seasons) on plasma 25 (OH)D3 concentrations.

Despite the all-year-round sunshine providing abundant UVB radiation, high prevalence of vitamin D deficiency (42%) and insufficiency (49%) in Ethiopian healthy adolescents and children was reported previously.[12,16] HIV patients and patients with active TB have lower levels of 25 (OH)D3 than healthy controls and patients with latent TB.[17,38] Accordingly, all patients who participated in this study had deficient (92%) or insufficient (8%) plasma 25 (OH)D3 levels at study enrollment, and none of them achieved a sufficient plasma 25 (OH)D3 level (>72.5nmol/L) during a 1-year cART follow-up period. The prevalence of vitamin D deficiency in our treatment-naive HIV-only and TB-HIV patients from Ethiopia is quite high compared to other reports from HIV patients in East Africa. Low prevalence of vitamin D insufficiency at baseline in TB-HIV co-infected (41%) and HIV (35%)-only infected patients from Uganda has been reported.[39] Likewise, only 9.2% and 43.6% of HIV patients from Tanzania were vitamin D deficient and -insufficient, respectively.[40] The finding of a high prevalence of SVDD in Ethiopian HIV patients might be because our study population consisted of very ill patients with low CD4 cell count (<200 cells/mm³) at baseline and a majority presented HIV stage 3 or 4 indicating progression to AIDS (Table 1). Association of nadir CD4 cell count (<200 cells/mm³) and HIV/TB disease progression with SVDD was reported previously.[25,37]

In our regression analysis (Table 4), low plasma 25 (OH)D3 and SVDD at baseline was significantly associated with high viral load, which is a marker for HIV disease progression.[41]
finding is in line with previous reports from the literature describing
association of vitamin D deficiency with HIV disease progression.\(^{14,15}\) Variations in the dietary source containing vitamin D may also contribute to between-population differences in vitamin D status. TB co-infected HIV patients had significantly lower levels of 25 (OH)D\(_3\) than patients without TB co-infection. Our finding further indicates that severe vitamin D deficiency in HIV patients is a risk factor for the development of active TB.\(^{16,17}\)

Treatment with rifampicin-based anti-TB treatment (up to 8 weeks) before the start of cART in TB-HIV co-infected patients resulted in no significant change in 25 (OH)D\(_3\) levels from baseline. However, a significant reduction of plasma 25 (OH)D\(_3\) levels was noted soon after initiating cART in both HIV-only and TB-HIV co-infected patients treated. The effect of efavirenz-based cART in lowering 25 (OH)D\(_3\) levels was more pronounced when given alone than when given together with rifampicin-based anti-TB therapy. In HIV only cohort, initiation of efavirenz-based cART increased the proportion of patients with SVDD by 3-fold at week 4 of cART, which persisted until week 16. As therapy continued, vitamin D levels were gradually restored, but 42% of the patients still had SVDD after 48 weeks of cART (Table 3). In contrast, in TB-HIV patients on anti-TB co-treatment initiation of efavirenz-based cART increased the proportion of patients with SVDD from 57% at baseline to 70% and 72% at 4 and 16 weeks of cART, respectively.

Previous in vitro studies suggested that a low 25 (OH)D\(_3\) level may be related to an increased catabolism of 25 (OH)D through CYP3A4 enzyme induction.\(^{18}\) In line with this, we found a significant correlation between CYP3A enzyme activity, as measured by 4- or 7-hydroxycholesterol to cholesterol ratio, and low 25 (OH)D concentration both at baseline and week 4 of cART. But as therapy continued, no significant association between CYP3A activity and vitamin D status was observed.

We previously reported a significant association between plasma efavirenz concentration and 4- or 7-hydroxycholesterol/cholesterol ratio in HIV patients.\(^{10}\) In the present study, we found a significant correlation between plasma efavirenz concentration and 25 (OH)D\(_3\) concentration. Vitamin D is involved in the regulation of CYP3A enzyme expression.\(^{19,20}\) CYP3A enzyme activity and expression are induced by 1,25-(OH)\(_2\)D\(_3\) via the vitamin D receptor.\(^{21}\) In addition, CYP3A catalyzes the 4- or 7-hydroxylation of 25 (OH)D\(_3\), and hence CYP3A induction may contribute to drug-induced vitamin D deficiency.\(^{18,19}\)

Rifaxipicin is a more potent inducer of CYP3A than efavirenz.\(^{14}\) Thus, it is plausible to expect a more pronounced effect of rifampicin in lowering the 25 (OH)D\(_3\) level compared to efavirenz-based cART alone. However, we found no significant effect of rifampicin-based anti-TB therapy (given before the initiation of cART) on the vitamin D status (Table 3). Based on our finding, the contribution of CYP3A enzyme induction by rifampicin per se may not be the major underlying mechanism, and perhaps other mechanism such as cholesterol-increasing effect of rifampicin may play a role to counterbalance the effect of efavirenz in modulating vitamin D status as discussed below. We found no significant correlations between CYP3A activity and vitamin D status at 16 and 48 weeks of cART. Probably, the importance of CYP3A catalyzed vitamin D metabolism may diminish, with the increased wellbeing of the patient during long-term cART. Indeed, improved CD4 recovery and vitamin D repletion in HIV patients on cART were reported recently. Consistent with our finding, 25 (OH)D\(_3\) levels have recently been shown to decline up to 24 weeks following initiation of efavirenz-based cART, but to stabilize thereafter.\(^{45}\)

Previously, we reported that initiation of efavirenz-based cART in HIV patients significantly lowers the plasma cholesterol level transiently.\(^{10}\) 7-dehydrocholesterol is a precursor to both cholesterol and vitamin D. Thus, efavirenz-based cART lowers the vitamin D level possibly by modulating the precursor concentration. To evaluate this, we monitored the change in plasma 25 (OH)D\(_3\) alongside the corresponding plasma cholesterol level in each patient before starting therapy and at different time points during treatment. Notably, TB-HIV co-infected patients had significantly lower pretreatment plasma concentration of both cholesterol and vitamin D compared to HIV-only patients. A similar pattern of change in plasma cholesterol level and vitamin D status over time was observed during cART, particularly when given alone (Fig. 3). Interestingly, initiation of efavirenz treatment significantly lowered both the plasma cholesterol and 25 (OH)D\(_3\) level dramatically in both treatment groups, but this effect was more pronounced when efavirenz-based cART was given alone than with rifampicin-based anti-TB co-treatment. Indeed, there was a significant positive correlation between plasma cholesterol level and vitamin D status during cART, particularly at week 4. Though not significant, a similar trend was observed at 16 and 48 weeks of cART. This may indicate that reduction in 25 (OH)D\(_3\) concentration by early initiation of efavirenz might be secondary to change in cholesterol concentration. In line with this, a recent in vitro study reported that treatment of cells with cholesterol resulted in a 3-fold increase in vitamin D relative to cholesterol synthesis, demonstrating that cholesterol feeds back via 7-dehydrocholesterol reductase (DHCR7) increasing vitamin D production.\(^{16}\) Indeed our results indicate that rifampicin-based anti-TB therapy alone significantly increased the plasma cholesterol level (Fig. 2). Thus, rifampicin-based anti-TB treatment might counterbalance the vitamin D-lowering effect of efavirenz to some extent by increasing the plasma cholesterol level and hence the vitamin D level in TB-HIV patients co-treated with rifampicin.

Increased plasma levels of 25 (OH)D\(_3\) after 1 year of cART has been reported previously in Japanese male HIV patients.\(^{17}\) Similarly, our study shows that at the 48th week of cART in HIV-only patients, both the plasma levels of 25 (OH)D\(_3\) and cholesterol levels restored gradually, which may be because of overall improved health status. In diverse HIV-infected populations, Vitamin D insufficiency and deficiency are associated with HIV disease progression, and virological failure after antiretroviral therapy initiation.\(^{44}\) Recent randomized clinical trials indicated that vitamin D supplementation improved CD4 recovery and vitamin D repletion suggesting potential benefit on immunologic recovery during cART.\(^{48,49}\)

### 4.1. Study Limitations

This study has some limitations. First, the study participants consisted of patients who had CD4 cell count <200 at baseline, following the WHO and Ethiopian national HIV treatment guideline valid during the study period. Thus, the result may not be directly extrapolatable to patients with high CD4 cell count at baseline. Second, since vitamin D status is influenced by genetic variations, darker skin, exposure to the sunshine, and dietary sources, the study result from a single study location and Ethiopian population may not be applicable for genetically diverse non-black populations living in the non-tropical zone.
5. Conclusions

The finding of this study suggests TB incidence and HIV disease progression are associated with low levels of 25 (OH)D3 at baseline. Early initiation of efavirenz-based ART is associated with low plasma 25 (OH)D3 levels and high incidence of SVDV, which sustains up to 16th week of cART. This effect is most pronounced when given alone than with rifampicin-based anti-TB co-treatment. As cART continues, 25 (OH)D3 levels restore gradually with time. Rifampicin-based anti-TB treatment initiated before cART has no significant effect on the 25 (OH)D3 levels. High plasma efavirenz concentration, high CYP3A activity, and before cART has no significant effect on the 25 (OH)D3 levels. Considering the very low levels of 25 (OH)D3 in both patient groups, supplementary vitamin D may be beneficial not only for TB-HIV patients, but also for HIV-only patients at the initiation of cART and anti-TB treatment.

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References

[1] Turnbull ER, Drobniowski F. Vitamin D supplementation: a comprehensive review on supplementation for tuberculosis prophylaxis. Expert Rev Respir Med 2015;9:269–75.
[2] Sudfeld CR, Wang M, Abous S, et al. Vitamin D and HIV progression among Tanzanian adults initiating antiretroviral therapy. PLoS One 2012;7:e49036.
[3] Klassen KM, Fairley CK, Chen M, et al. Vitamin D deficiency may be associated with a more rapid decline in CD4 cell count to <350 cells/μl in untreated HIV-infected adults. Curr HIV Res 2015;13:517–23.
[4] Calvo MS, Whiting SJ, Barton CN. Vitamin D intake: a global comprehensive review on supplementation for tuberculosis prophylaxis. Expert Rev. Nutr 2014;3:274–9.
[5] Holick MF, Binkley NC, Bischoff-Ferrari HA, et al. Vitamin D deficiencies in humans and animals. Endocr Rev 2011;32:1183–217.
[6] Brown TT, McComsey GA. Association between initiation of antiretroviral therapy with efavirenz and decreases in 25-hydroxyvitamin D. Antivir Ther 2010;15:425–9.
[7] Conrado T, Miranda-Filho Dde B, Ximenes RA, et al. Vitamin D deficiency and its effect on plasma exposure in HIV patients. Clin Pharmacol Ther 2011;89:84–9.
[8] Bolland MJ, Chiu WW, Davidson JS, et al. The effects of seasonal variation in serum 25-hydroxyvitamin D among a West African population of tuberculosis patients and unmatched healthy controls. Am J Clin Nutr 2007;86:1376–83.
[9] Klingberg E, Olerod G, Konar J, et al. Evidence for induction of ABC transporters in peripheral blood mononuclear cells in humans after 14 days of efavirenz treatment. Antimicrob Agents Chemother 2014;58:7523–9.
[10] Habtewold A, Amogne W, Makonnen E, et al. Pharmacogenetic & pharmacokinetic aspects of CYP3A4 induction by efavirenz in HIV patients. Pharmacogenomics J 2013;13:484–9.
[11] Lawn SD, Meinert G, Melleron H, et al. Management of HIV-associated tuberculosis in resource-limited settings: a state-of-the-art review. BMC Med 2013;11:253.
[12] Yimer G, Ueda N, Habtewold A, et al. Pharmacogenetic & Pharmacokinetic Biomarker for Efavirenz Based ARV and Rifampicin Based Anti-TB Drug Induced Liver Injury in TB-HIV Infected Patients. PLoS One 2011;6:e27810.
[13] Mukonzo JK, Okwera A, Nakasujja N, et al. Pharmacogenetic & pharmacokinetic aspects of CYP3A4 induction by efavirenz in primary human hepatocytes: comparison with rifampin and phenobarbital. J Clin Pharmacol 2004;44:1273–81.
[14] Ngyansia E, Mugusi S, Minzi O, et al. Effect of Rifampicin and CYP2B6 Genotype on Long Term Efavirenz Autoinduction and Plasma Exposure in HIV Patients With or Without Tuberculosis. Clin Pharmacol Ther 2011;90:496–13.
[15] Schmiedlin-Ren P, Thummler KE, Fisher JM, et al. Expression of enzymatically active CYP3A4 by Caco-2 cells grown on extracellular matrix-coated permeable supports in the presence of 1alpha,25-dihydroxyvitamin D3. Mol Pharmaco 1997;51:741–54.
[16] Drocourt L, Ourlin JC, Pascussi JM, et al. Expression of CYP3A4, CYP2B6, and CYP2C9 is regulated by the vitamin D receptor pathway in primary human hepatocytes. J Biol Chem 2002;277:3125–32.
[17] Wang Z, Lin YS, Zheng XF, et al. An inducible cytochrome P450 3A4-dependent vitamin D catalytic pathway. Mol Pharmacol 2012;81:498–509.
[18] Wang Z, Schuetz EG, Xu Y, et al. Interplay between vitamin D and the drug metabolizing enzyme CYP3A4. J Steroid Biochem Mol Biol 2013;136:54–8.
[19] Habtewold A, Amogne W, Makonnen E, et al. Long-term effect of efavirenz autoinduction on plasma/peripheral blood mononuclear cell drug exposure and CD4 count is influenced by UGT2B7 and CYP2B6 genotypes among HIV patients. J Antimicrob Chemother 2011;66:2350–61.
[20] Ngyansia E, Mugusi S, Minzi O, et al. Long-term efavirenz autoinduction and its effect on plasma exposure in HIV patients. Clin Pharmacol Ther 2010;88:676–84.
[21] Ngyansia E, Minzi O, Mugusi S, et al. Pharmacokinetic and pharmacogenomic modeling of the CYP3A4 activity marker 4beta-hydroxycholesterol during efavirenz treatment and efavirenz/rifampicin co-treatment. J Antimicrob Chemother 2013;68:484–9.
[22] Mukonzo JK, Owen JS, Ogwal-Okeng J, et al. Pharmacogenetic-based efavirenz dose modification: suggestions for an African population and the drug PLCO2B6 genotypes. PLoS One 2014;9:e86919.
[23] Mukonzo JK, Namugya S, Waako P, et al. CYP2B6 genotype, but not rifampicin-based anti-TB cotreatments, explains variability in long-term efavirenz plasma exposure. Pharmacogenomics 2014;15:1423–35.
[24] Welz T, Childs K, Ibrahim F, et al. Efavirenz is associated with severe vitamin D deficiency and increased alkaline phosphatase. AIDS 2010;24:1923–8.
[25] Habtewold A, Makonnen E, Amogne W, et al. Is there a need to increase the dose of efavirenz during concomitant rifampicin-based antituberculo-losis therapy in sub-Saharan Africa? The HIV-TB pharmacogen study. Pharmacogenomics 2015;16:1047–64.
[26] Holick MF, Binkley NC, Bischoff-Ferrari HA, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab 2011;96:1911–30.
[27] Nylen H, Björkhagen-Bergman L, Ekstrom L, et al. Plasma levels of 25-hydroxyvitamin D3 and in vivo markers of cytochrome P450 3A activity in Swedes and Koreans: effects of a genetic polymorphism and oral contraceptives. Basic Clin Pharmacol Toxicol 2014;113:366–71.
[28] Burhenne J, Matteie AK, Passakova I, et al. No evidence for induction of ABC transporters in peripheral blood mononuclear cells in humans after 14 days of efavirenz treatment. Antimicrob Agents Chemother 2010;54:4183–91.
[29] Schöningberg E, Olerod G, Konar J, et al. Seasonal variations in serum 25-hydroxy vitamin D levels in a Swedish cohort. Endocrinology 2015;149:800–8.
[30] Bolland MJ, Chiu WW, Davidson JS, et al. The effects of seasonal variation of 25-hydroxyvitamin D on diagnosis of vitamin D insufficiency. N Z Med J 2008;121:63–74.
[31] Holick MF. MicCollum Award Lecture, 1994: vitamin D-new horizons for the 21st century. Am J Clin Nutr 1994;60:619–30.
[32] Wejse C, Olesen R, Rambia P, et al. Serum 25-hydroxyvitamin D in a West African population of tuberculosis patients and unmatched healthy controls. Am J Clin Nutr 2007;86:1376–83.
[33] Gebrezgabiher T, Stoecker BJ. Vitamin D insufficiency in a sunshine-sufficient area: southern Ethiopia. Food Nutr Bull 2013;34:829–33.
[34] Nakasujja N, Ngyansia E, Amogne W, et al. Vitamin D deficiency and its predictors in a country with thirteen months of sunshine: the case of school children in central Ethiopia. PLoS One 2015;10:e0120963.
[35] Herradore Z, Sordo L, Gadisa E, et al. Micronutrient deficiencies and related factors in school-aged children in Ethiopia: a cross-sectional study in Libo Kemkem and Fogera districts, Amhara regional State. PLoS One 2014;9:e112858.
[36] Theodorou M, Serste T, Van Gossum M, et al. Factors associated with vitamin D deficiency in a population of 2044 HIV-infected patients. Clin Nutr 2014;33:274–8.
[37] Venturini E, Facchini L, Martinez-Alier N, et al. Vitamin D and tuberculosis: a multicenter study in children. BMC Infect Dis 2014;14:652.
[38] Conesa-Botella A, Gosoaverts O, Massinga-Loembe M, et al. Low prevalence of vitamin D deficiency in Ugandan HIV-infected patients with and without tuberculosis. Int J Tuberc Lung Dis 2016;19:1517–21.
Sudfeld CR, Giovannucci EL, Isanaka S, et al. Vitamin D status and incidence of pulmonary tuberculosis, opportunistic infections, and wasting among HIV-infected Tanzanian adults initiating antiretroviral therapy. J Infect Dis 2013;207:378–85.

Ghani AC, de Wolf F, Ferguson NM, et al. Surrogate markers for disease progression in treated HIV infection. J Acquir Immune Defic Syndr 2001;28:226–31.

Viard JP, Souberbielle JC, Kirk O, et al. Vitamin D and clinical disease progression in HIV infection: results from the EuroSIDA study. AIDS 2011;25:1305–15.

Arnedo-Pena A, Juan-Cerdan JV, Romeu-Garcia A, et al. Vitamin D status and incidence of tuberculosis among contacts of pulmonary tuberculosis patients. Int J Tuberc Lung Dis 2015;19:65–9.

Wang K, Chen S, Xie W, et al. Retinoids induce cytochrome P450 3A4 through RXR/VDR-mediated pathway. Biochem Pharmacol 2008;75:2204–13.

Nylén et al. Medicine (2016) 95:34

Havers F, Smeaton L, Gupte N, et al. 25-Hydroxyvitamin D insufficiency and deficiency is associated with HIV disease progression and virological failure post-antiretroviral therapy initiation in diverse multinational settings. J Infect Dis 2014;210:244–53.

Prabhu AV, Liu W, Sharpe LJ, et al. Cholesterol-Mediated Degradation of 7-Dehydrocholesterol Reductase Switches the Balance from Cholesterol to Vitamin D Synthesis. J Biol Chem 2016.

Koga I, Seo K, Yoshino Y, et al. Increase of 25-hydroxyvitamin D levels after initiation of combination antiretroviral therapy. J Infect Chemother 2015;21:737–41.

Coelho L, Cardoso SW, Luz PM, et al. Vitamin D3 supplementation in HIV infection: effectiveness and associations with antiretroviral therapy. Nutr J 2015;14:81.

Steenhoff AP, Schall JL, Samuel J, et al. Vitamin D (3) supplementation in Botswana children and adults with HIV: a pilot double blind randomized controlled trial. PLoS One 2015;10:e0117123.