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Vitamin D and HIV Progression among Tanzanian Adults Initiating Antiretroviral Therapy

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Abstract

Background: There is growing evidence of an association between low vitamin D and HIV disease progression; however, no prospective studies have been conducted among adults receiving antiretroviral therapy (ART) in sub-Saharan Africa.

Methods: Serum 25-hydroxyvitamin D (25(OH)D) levels were assessed at ART initiation for a randomly selected cohort of HIV-infected adults enrolled in a trial of multivitamins (not including vitamin D) in Tanzania during 2006–2010. Participants were prospectively followed at monthly clinic visits for a median of 20.6 months. CD4 T-cell measurements were obtained every 4 months. Proportional hazard models were utilized for mortality analyses while generalized estimating equations were used for CD4 T-cell counts.

Results: Serum 25(OH)D was measured in 1103 adults 9.2% were classified as vitamin D deficient (<20 ng/ml), 43.6% insufficient (20–30 ng/mL), and 47.2% as sufficient (>30 ng/mL). After multivariate adjustment, vitamin D deficiency was significantly associated with increased mortality as compared to vitamin D sufficiency (HR: 2.00; 95% CI: 1.19–3.37; p = 0.009), whereas no significant association was found for vitamin D insufficiency (HR: 1.24; 95% CI: 0.87–1.78; p = 0.24). No effect modification by ART regimen or change in the associations over time was detected. Vitamin D status was not associated with change in CD4 T-cell count after ART initiation.

Conclusions: Deficient vitamin D levels may lead to increased mortality in individuals receiving ART and this relationship does not appear to be due to impaired CD4 T-cell reconstitution. Randomized controlled trials are needed to determine the safety and efficacy of vitamin D supplementation for individuals receiving ART.

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Introduction

In 2002 only 2% of HIV-infected individuals eligible for antiretroviral therapy (ART) in sub-Saharan were receiving treatment, while by the end of 2010 this proportion increased to 49% with over 5 million people receiving ART [1]. Despite successes in expanding treatment coverage, individuals initiating ART in sub-Saharan Africa experience high mortality rates and interventions are needed to prolong and improve quality of life [2,3].

Vitamin D is a potent immunomodulator with effects on both adaptive and innate immune responses [4,5]. Vitamin D may play a particularly strong role in control of intracellular pathogens by enhancing cell-mediated immunity, production of antimicrobial peptides, and phagocytic activity of macrophages [6–9]. As a result, HIV-infected individuals with adequate levels of vitamin D may better control HIV or opportunistic infections compared to deficient individuals [10]. Further, vitamin D has effects on multiple organ systems and HIV-infected individuals with low levels of vitamin D could experience increased complications of ART including: cardiovascular disease, insulin resistance, and renal impairment [11–13].

Three previous cohorts have investigated the association of vitamin D status and HIV mortality; however, each of these studies has methodological or generalizability limitations. The first small study conducted in Norway before introduction of ART found that HIV-infected individuals with low 1, 25$(OH)_2$D (active form of vitamin D) had significantly decreased survival time compared to individuals with normal levels [14]. However, serum 1,25$(OH)_2$D is considered a sub-optimal measure of vitamin D status and serum 25-hydroxyvitamin D (25(OH)D) is the only vitamin D metabolite that should be employed to assess vitamin D

a result we analyzed 25(OH)D levels categorically with clinical cut-offs as well as continuously [29,30]. We defined vitamin D deficiency as 25(OH)D concentrations <20 ng/mL, insufficiency 20–30 ng/mL, and sufficiency as >30 ng/mL [31–33]. The possible non-linear relation between serum 25(OH)D and mortality was also examined non-parametrically with restricted cubic splines [34,35]. Tests for non-linearity used the likelihood ratio test, comparing the model with only the linear term to the model with the linear and the cubic spline terms. Individuals without events were censored at the date of last follow-up visit. Confounders considered for multivariate mortality models included variables with univariate p-values <0.20 in the risk factor analysis or variables noted to be significant confounders in previous studies were selected for inclusion.

Associations of vitamin D status and CD4 T-cell counts were analyzed with generalized estimating equations. Change in CD4 T-cell count between consecutive visits was treated as a longitudinal continuous outcome and vitamin D, time since ART initiation, along with other baseline covariates as explanatory variables. We used an m-dependent working correlation matrix (m = 1) assuming the correlation coefficient of adjacent observations are non-zero and equal, and robust estimators of the variances were utilized to construct confidence intervals. The robust estimators are consistent estimators of the variances even if the working correlation matrix is misspecified. The potential non-linear relationship of change in T-cell counts between consecutive visits over time was examined non-parametrically with restricted cubic splines for time since ART initiation [34,35]. If a non-linear relationship was found, we added the selected cubic spline terms to the above-specified model as covariates. To assess whether vitamin D status was associated with change in CD4 T-cell between consecutive visits over time, we included the interactions of vitamin D status with time since ART initiation and its splines. The robust score test was used to determine whether vitamin D deficient and insufficient individuals differed in CD4 T-cell trajectory as compared to sufficient individuals. Confounders were also selected from the risk factor analysis; however, baseline CD4 T-cell count was not adjusted for as it will result in biased estimates [12].

Effect modification by all covariates, randomized multivitamin regimen, and ART regimen were considered for all analyses. Interaction by ART regimen was examined for each of the four regimen combinations and also by antiretroviral drug (i.e. efavirenz-containing regimen). To determine whether effect modification was statistically significant, we used the likelihood ratio test in proportional hazard models and the robust score test in GEE analyses. Missing data for covariates was retained in the analysis using the missing indicator method for variables missing greater than 1% of the observations [36]. All p-values were 2-sided and $p<0.05$ considered statistically significant. Statistical analyses were performed using the SAS v 9.2 (SAS Institute Inc., Cary, NC, USA).

Ethics Statement

Written informed consent was obtained from all participants included in the parent trial. The trial protocol was approved by the institutional review boards of the Harvard School of Public Health, Muhimbili University of Health and Allied Sciences, Tanzania Food and Drug Authority and National Institute of Medical Research.

Results

A total of 1103 individuals were randomly selected for 25(OH)D testing from 3,418 individuals enrolled in the parent trial. There were no significant differences between the randomly selected cohort and the trial population. 101 (9.2%) individuals were classified as vitamin D deficient (<20 25(OH)D ng/mL), 481 (43.6%) as insufficient (20–30 ng/mL), and 521 (47.2%) as vitamin D sufficient (>30 ng/mL). Baseline characteristics of the cohort at ART initiation visit are presented in Table 1. A multivariate risk factor analysis determined low vitamin D (25(OH)D <30 ng/mL) was independently associated with younger age ($p<0.001$), increased CD4 T-cell count ($p<0.001$), and season of the ART initiation visit (Table 2).

The median follow-up time was 20.6 months (IQR: 8.4–33.8), during which 151 deaths were recorded. A crude Kaplan-Meir curve for all-cause mortality by vitamin D status is presented in Figure 1 showing that at 24 months post ART initiation 22.8% of the individuals with deficient vitamin D levels died as compared to 14.1% and 13.1% of individuals with insufficient and deficient levels of vitamin D, respectively.

The results of univariate and multivariate proportional hazard analyses for mortality outcomes by vitamin D status are presented in Table 3. After multivariate adjustment, the hazard of death was 2.00 (95% CI: 1.19–3.37; p = 0.009) times greater for individuals with vitamin D deficiency at baseline compared to vitamin D sufficient individuals. There was no significant increase for individuals with insufficient levels of vitamin D compared to sufficient individuals (HR: 1.24; 95%: 0.87–1.78; p = 0.240). Further, when analyzing 25(OH)D as a continuous variable we found a non-linear relationship with increasing risk of all-cause mortality below 25 ng/mL and seemingly no increased benefit to baseline vitamin D levels greater than 30 ng/mL (Figure 2). No significant effect modification by baseline CD4 T-cell count, age, sex, randomized multivitamin regimen, ART regimen or specific antiretroviral drug was found. There was also no indication that the hazard of death for vitamin D deficiency (p = 0.97) or insufficiency (p = 0.40) changed over time.

A total of 875 individuals had 2 or more CD4 T-cell count measurements during the study period. As expected the change in CD4 T-cell count post ART initiation was non-linear. Participants, regardless of vitamin D status, experienced dramatic increases in CD4 T-cell counts during the first 6 months of ART with more modest gains thereafter (Figure 3). In crude models adjusting solely for time between visits, the trajectory of change in CD4 T-cell count between consecutive visits did not differ in vitamin D deficient (p = 0.403) or insufficient (p = 0.885) individuals as compared to those with sufficient vitamin D levels at baseline. There were no differences in CD4 T-cell trajectory in multivariate models further adjusting for sex, age, WHO HIV disease stage, BMI, and ART regimen for deficient (p = 0.374) or insufficient individuals (p = 0.943) as compared to sufficient individuals. No significant effect modification by age, sex, randomized multivitamin regimen, ART regimen or specific antiretroviral drug was found.

Discussion

In this study we found individuals with deficient levels of vitamin D (<20 ng/mL) at ART initiation had significantly increased risk of mortality as compared to individuals with sufficient levels of vitamin D (>30 ng/mL), whereas there was no significant increase for individuals with vitamin D insufficiency (20–30 ng/mL). The benefit of sufficient vitamin D does not appear to be mediated through improved immune reconstitution as there was no association between vitamin D status and change in CD4 T-cell count after ART initiation. We also found no effect

modification by any factor, including ART regimen, in either mortality or CD4 T-cell analyses.

The mortality results of this study are consistent with previous cohort studies. In the EUROSIDA study adults in the lowest tertile of 25(OH)D (<12 ng/mL) had significantly increased incidence of mortality as compared adults in the middle (12.1–20 ng/mL) and highest tertile (≥20 ng/mL), but there was no significant difference between the middle and highest tertiles [21]. Individuals in the EUROSIDA cohort had markedly low levels of vitamin D with 89% of individuals below 30 ng/mL and as a result, the study likely had inadequate power to examine the relationship using clinical classifications with sufficiency (>30 ng/mL) defining the referent group. The second cohort study consisting of Tanzanian pregnant women did not find a significant association of low vitamin D with all-cause mortality using a cut-off of 32 ng/mL, but found a significant difference when comparing women in the lowest quintile of vitamin D to the highest quintile [16]. As opposed to the EUROSIDA study, these Tanzanian pregnant women may have had too high levels of vitamin D to find a significant difference with these clinical cutoffs since only 40% of women had levels below 32 ng/mL with very few below 20 ng/mL. Our categorical analyses in this study found individuals with vitamin D deficiency (<20 ng/mL) had significantly increased risk of mortality as compared to those with sufficient vitamin D levels (≥30 ng/mL), while individuals with vitamin D insufficiency (20–30 ng/mL) appeared to have more moderately elevated mortality risk, but results were not statistically significant. Nevertheless, our analysis of 25(OH)D as a continuous exposure suggests mortality risk begins to increase at levels below 25 ng/mL. Accordingly, using the standard vitamin D status classifications (<20, 20–30, ≥30 ng/mL) may make cross

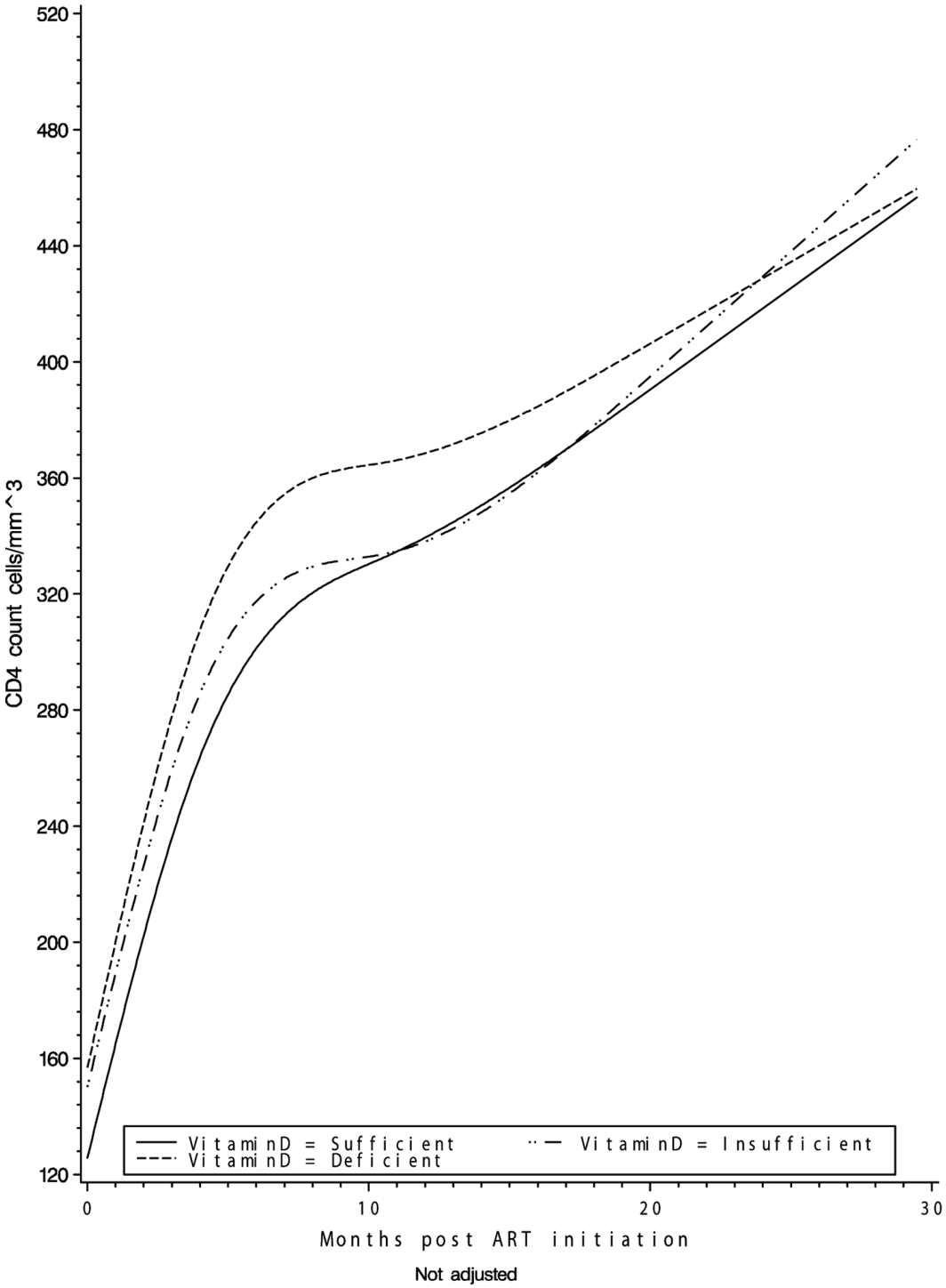

Figure 3. Crude CD4 T-cell count overtime by vitamin D status.
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found low vitamin D was significantly associated with decreased change in CD4 T-cell count (current minus nadir) overtime [48]. Nevertheless, the authors adjusted for nadir CD4 T-cell count in analyses, which can produce bias if vitamin D is associated with baseline health status and also vitamin D was measured at least 24 weeks after start of ART which increases the risk of reverse causation [49]. Further, two small randomized controlled trials in HIV-infected children on ART also reported no effect of vitamin D supplementation on change in CD4 T-cell count overtime [50,51]. Overall, our risk factor analysis and other cross-sectional studies have produced varying results for the impact of vitamin D on CD4 T-cell count, but our prospective analysis along with other longitudinal studies and randomized trials suggest there is no pronounced difference in change in CD4 T-cell counts during the initial months of ART by vitamin D status.

There may also be immunomodulatory benefits to maintaining sufficient vitamin D for other aspects of adaptive immune responses. In addition to IL-2 suppression, vitamin D also reduces production of other proinflammatory Th1 and Th17 cytokines, while also enhancing production of Th2 promoting cytokines including: IL-4, IL-5, and IL-10 [52–55]. $1,25(OH)_2D$ has also been shown to reduce B-cell proliferation, plasma differentiation, and immunoglobulin production *in vitro* [56,57]. Further, $1,25(OH)_2D$ can also suppress the response of Th17 cells, which cause inflammation and tissue destruction and also induce Foxp3+ T regulatory cells, which suppress excessively strong CD4 T-cell proliferation [58,59]. The sum of these actions will likely decrease inflammation, tissue damage, and reduce excessively strong immune responses to opportunistic infections in individuals with adequate levels of vitamin D which may in turn lead to reduced mortality [60,61]. Overall, there are multiple mechanisms by which vitamin D may support innate or adaptive immune responses to opportunistic infections and reduce subsequent mortality.

Antiretroviral drugs can alter vitamin D metabolism and result in decreased circulating 25(OH)D [19]. The non-nucleoside reverse transcriptase inhibitor (NNRTI) efavirenz induces 24-hydroxylase, which hydrolyzes active vitamin D to its inactive form [62]. A prospective study of individuals initiating ART in the US found individuals receiving regimens containing efavirenz had 1.8 times the risk of developing 25(OH)D levels $\leq$15 ng/mL as compared to individuals initiating regimens containing protease inhibitors [63]. Further, the Monet trial found that patients who switched from regimens containing efavirenz and/or zidovudine to darunavir/ritonavir experienced increases in serum 25(OH)D [64]. Protease inhibitors have also been noted to alter vitamin D metabolism, but we were unable to investigate this class of antiretroviral drugs as they were not used in Tanzania during the study period [65,66]. We did not find effect modification of mortality or CD4 T-cell associations by efavirenz-containing or other ART regimens in this analysis. However, we may have had limited statistical power to detect multiplicative interaction, and it is plausible that vitamin D insufficient individuals who decrease serum 25(OH)D to deficient levels as a result of ART or use of particular antiretroviral drugs may experience slightly increased mortality relative to those on ART therapy who maintain sufficient serum 25(OH)D levels.

A major strength of this study is the use of clinically defined vitamin D cut-offs, which can make direct comparison of studies easier. We also quantified 25(OH)D with HPLC-MS/MS, a reference method, which will reduce non-differential misclassification of vitamin D status or even bias if individuals with more severe HIV disease produce metabolites or other molecules which may interfere with 25(OH)D measurement using radioimmunoassy (RIA) [15,67] Furthermore, measuring 25(OH)D at ART initiation allows for examination of effect modification by ART regimen. It is unlikely interaction would be detected in individuals already receiving ARTs at the time of vitamin D measurement, since individuals would have already experienced a reduction in circulating 25(OH)D as a result of treatment.

The study also has several important limitations. First, 25(OH)D was measured at a single time point and we are unable to determine whether deficient vitamin D levels at a single time point or long term deficiency is biologically relevant. Nevertheless, we found no change in the association of vitamin D deficiency at baseline and mortality over time, which suggests a single vitamin D measurement at ART initiation may be able to indicate which patients may benefit from vitamin D supplementation. Second, the results of this study may not be directly generalizable to other HIV-infected populations, including pregnant women or children on ART. A previous study in HIV-infected pregnant women not receiving ART in Tanzania found having vitamin D levels above 32 ng/mL provided additional benefit; however, this study indicated no benefit to having levels above 30 ng/mL for men and non-pregnant women on ART in the same setting [17]. Further, we presented an observational study from which reverse causation cannot be ruled out as advanced HIV disease or other co-morbidities may have led to lower vitamin D levels. We controlled multivariate analyses for multiple indicators of disease severity (CD4 T-cell count, WHO HIV disease stage, BMI) and if residual confounding by these factors is present we may actually underestimate the association of vitamin D and mortality. Nevertheless, unmeasured confounding by other factors like tuberculosis or hepatitis C infection at baseline may still produce biased results [68,69].

This study suggests that deficient vitamin D levels may lead to increased mortality in HIV-infected men and non-pregnant women receiving ART and this association does not appear to be mediated by impaired CD4 T-cell reconstitution. However, due to the observational design of this study a causal relationship cannot be concluded. Accordingly, randomized controlled trials are warranted to determine the safety and efficacy of vitamin D supplementation for HIV-infected individuals receiving ART.

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Author Contributions

Conceived and designed the experiments: SA FM WWF. Analyzed the data: CRS MW. Wrote the paper: CRS MW SA ELG FMM WWF. Developed the data analysis plan: CRS MW ELG WWF.

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