MAJOR ARTICLE

Vitamin D Status and Incidence of Pulmonary Tuberculosis, Opportunistic Infections, and Wasting Among HIV-Infected Tanzanian Adults Initiating Antiretroviral Therapy

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(See the editorial commentary by Martineau on pages 373–5.)

Background. Maintaining vitamin D sufficiency may decrease the incidence of pulmonary tuberculosis and other infectious diseases. We present the first prospective study of vitamin D among human immunodeficiency virus (HIV)–infected adults receiving antiretrovirals in sub-Saharan Africa.

Methods. Serum 25-hydroxyvitamin D (25(OH)D) level was assessed at antiretroviral therapy (ART) initiation for 1103 HIV-infected adults enrolled in a trial of multivitamins (not including vitamin D) in Tanzania. Participants were prospectively followed at monthly visits at which trained physicians performed a clinical examination and nurses took anthropometric measurements and assessed self-reported symptoms. Cox proportional hazards models estimated hazard ratios (HRs) of morbidity outcomes.

Results. After multivariate adjustment, vitamin D deficiency (defined as a concentration of <20 ng/mL) had a significantly greater association with incident pulmonary tuberculosis, compared with vitamin D sufficiency (HR, 2.89; 95% confidence interval [CI], 1.31–7.41; $P = .027$), but no association was found for vitamin D insufficiency (defined as a concentration of 20–30 ng/mL; $P = .687$). Deficiency was also significantly associated with incident oral thrush (HR, 1.96; 95% CI, 1.01–3.81; $P = .046$), wasting (HR, 3.10; 95% CI, 1.33–7.24; $P = .009$), and >10% weight loss (HR, 2.10; 95% CI, 1.13–3.91; $P = .019$). Wasting results were robust to exclusion of individuals experiencing pulmonary tuberculosis. Vitamin D status was not associated with incident malaria, pneumonia, or anemia.

Conclusions. Vitamin D supplementation trials for adults receiving ART appear to be warranted.

Keywords. Vitamin D; micronutrient; HIV; tuberculosis; wasting; malaria; pneumonia; morbidity; ART.

Successful antiretroviral therapy (ART) for human immunodeficiency virus (HIV)–infected individuals reduces viral load, restores host immune responses, and provides protection against opportunistic infections [1, 2]. HIV-infected individuals in sub-Saharan Africa often initiate ART at low $CD4^+$ T-cell counts and as a result are at high risk for opportunistic infections and death during the significant amount of time needed to reconstitute their immune systems [3, 4]. Accordingly, interventions that hasten $CD4^+$ T-cell recovery or promote well-regulated and robust immune responses may prolong and improve quality of life for individuals receiving ART.

Vitamin D may be such an intervention because of its potent immunomodulatory effects on both adaptive and innate immune responses [5, 6]. By using data from a prospective cohort study, we recently reported

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that vitamin D deficiency was associated with increased hazard of death but not change in $CD4^+$ T-cell count over time among adults initiating ART in Tanzania [7]. Consequently, the relationship between vitamin D and mortality may be mediated by decreased incidence or severity of opportunistic infections. In a previous cohort study of pregnant women in Tanzania not receiving ART, low vitamin D levels (<30 ng/mL) were associated with increased incidence of acute upper respiratory tract infections, oral thrush, and wasting [8]; however, the role of vitamin D may be modified during immune reconstitution with ART, and results in pregnant women may not be directly generalizable to men and nonpregnant women.

Multiple case-control studies have investigated the association of vitamin D status and susceptibility to active tuberculosis, with the majority of these studies documenting significantly lower serum 25-hydroxyvitamin D (25(OH)D) levels in individuals with active tuberculosis as compared to healthy controls or general population [9–14]. Nevertheless, 25(OH)D may be reduced in individuals with inflammation or immune activation, and the observed cross-sectional findings may be attributed to reverse causation with *Mycobacterium tuberculosis* infection, leading to reduced 25(OH)D [15]. Only 1 prospective cohort study, using a design that may reduce the risk of reverse causation, has been published, and it involved a Pakistani cohort of HIV-uninfected household contacts of tuberculosis patients [16]. This study found that household contacts with vitamin D deficiency had significantly increased risk of developing active tuberculosis within 4 years as compared to household contacts with sufficient levels of 25(OH)D [16]. One randomized trial of vitamin D supplements and tuberculosis incidence has also been conducted in HIV-uninfected Mongolian children [17], which found that children who were supplemented with vitamin D had a nonsignificant reduction in tuberculin skin test (TST) conversion during 6 months of follow-up as compared to those supplemented with placebo. Regardless, the results of these longitudinal studies may not be generalizable to HIV-infected adults, and TST conversion results may not directly translate to prevention of active tuberculosis.

To address the need for longitudinal data among HIV-infected individuals receiving ART, here we present morbidity findings from the prospective observational cohort study involving the same adult cohort as our previous vitamin D and mortality work. In this study, we examine the association of vitamin D and incident pulmonary tuberculosis, pneumonia, malaria, oral thrush, wasting, weight loss, and other comorbidities.

METHODS

Study Population

This prospective cohort study consisted of a randomly selected sample of HIV-infected men and women initiating ART who were enrolled in the Trial of Vitamins and HAART in HIV Disease Progression, conducted in Dar es Salaam, Tanzania, during 2006–2009 (Clinicaltrials.gov NCT00383669) [18]. This trial was a double-blind, randomized controlled trial assessing the effect of daily oral supplements of vitamins B-complex, C, and E, at high versus standard levels of the recommended dietary allowance, on HIV disease progression or death. Individuals were eligible for the study if they were aged ≥18 years, HIV infected, initiated ART at enrollment, and intended to stay in Dar es Salaam for at least 2 years. Women who were pregnant or lactating were excluded from the study. At the time of the study, the Tanzanian national treatment guideline recommended initiation of highly active antiretroviral therapy for patients with World Health Organization (WHO) HIV disease stage IV, patients with a $CD4^+$ T-cell count of <200 cells/μL, and patients with WHO HIV stage III disease and a $CD4^+$ T-cell count <350 cells/μL [19]. First-line drug combinations included stavudine (d4T), lamivudine (3TC), nevirapine (NVP), zidovudine (AZT), and efavirenz (EFV). AZT was substituted for d4T for individuals who had peripheral neuropathy or could not tolerate d4T. EFV was substituted for NVP in patients who could not tolerate NVP. Cotrimoxazole prophylaxis was provided when $CD4^+$ T-cell counts were <200 cells/μL, and treatment for all opportunistic infection was provided according to the national and WHO guidelines.

Baseline Covariate Assessment

A total of 3418 individuals consented and were enrolled into the parent trial. At enrollment, a full clinical examination was conducted, and a structured interview was completed to collect information on demographic characteristics. Study physicians performed a complete medical examination, during which HIV disease stage was assessed in accordance with the WHO guidelines, and blood specimens were collected at baseline and every 4 months thereafter for determination of absolute $CD4^+$ T-cell counts (FACSCalibur flow cytometer, Becton Dickinson, San Jose, CA) and complete blood counts (AcT5 Diff AL analyzer, Beckman Coulter, Miami, FL). Height and weight were measured by trained research nurses, using standardized procedures.

Vitamin D Assessment

A total of 1105 participants, of which 1103 (99.8%) had samples available, were randomly selected to have vitamin D levels measured at baseline. 25(OH)D, the storage form of vitamin D in serum, was quantified by high performance liquid chromatography tandem mass spectrometry using an API-5000 (AB Sciex, Foster City, CA) at Children's Hospital Boston, as described elsewhere [7, 20]. Briefly, serum samples were first extracted and centrifuged, and the supernatant was injected into the Aria-TLX-2, passed through a Cyclone-P

column (Thermo Fisher Scientific), and then eluted through a Kinetex C column (Phenomenex, Torrance, CA). The eluate then underwent atmospheric pressure chemical ionization and was passed through a triple quadrupole mass spectrometer for detection and quantified measurements. Day-to-day precision (defined as the coefficient of variation expressed as a percentage) at various levels of 25(OH)D ranged from 5.6% to 8.5%.

Outcome Assessment and Definitions

Participants were followed at monthly clinic visits during which study physicians performed a clinical examination. Pneumonia and oral thrush were diagnosed on the basis of symptoms reported by patients and signs assessed by study physicians. Malaria diagnosis was defined as presentation of symptoms consistent with malaria, with confirmation by observation of any parasitemia in a blood smear. Pulmonary tuberculosis was diagnosed according to Tanzanian National Tuberculosis and Leprosy Programme guidelines. Participants with symptoms of pulmonary tuberculosis were requested to provide a spot sputum specimen at the study visit during which symptoms were noted, an early morning specimen before a second clinic visit scheduled for the next day, and a third sputum specimen at the second clinic visit. Individuals received a diagnosis of pulmonary tuberculosis if at least 1 of the 3 sputum smears was positive for acid-fast bacilli, using Ziehl-Nielsen staining techniques, or when a chest radiograph showed features consistent with tuberculosis in the absence of positive sputum smear results [21].

A nurse also assessed self-reported signs and symptoms since the previous visit at each monthly clinic visit, including fatigue, nausea, diarrhea, skin rashes or lesions, peripheral neuropathy, and genital discharge or ulcers. Height and weight were also measured by study nurses at each monthly clinic visit. Wasting was defined as a body mass index (BMI; calculated as the weight in kilograms divided by the height in meters squared) of <18.5 [22]. Weight loss of >10% was also selected as an outcome, on the basis of the definition of HIV-related wasting [23]. Hemoglobin levels were assessed in blood samples collected every 4 months. Anemia was defined as a hemoglobin level of <130 g/L, for men, and <120 g/L, for women, whereas severe anemia was defined as a hemoglobin level of <85 g/L, for both sexes [24, 25].

Statistical Methods

We defined vitamin D deficiency as a 25(OH)D concentration of <20 ng/mL; insufficiency, as a concentration of 20–30 ng/mL; and sufficiency, as a concentration of >30 ng/mL [26, 27]. Proportional hazard models were used to investigate the relationship of vitamin D levels at baseline and first occurrence of pneumonia, malaria, oral thrush, pulmonary tuberculosis, severe anemia, anemia, wasting, and >10% weight loss [28]. The results of these analyses are reported as adjusted hazard ratios (HRs) and their 95% confidence intervals (CIs). We excluded individuals who received a diagnosis of the event of interest at baseline, as well as those experienced the event within 1 month of the baseline visit, to reduce the risk of reverse causation. Individuals without events were censored at the date of the last follow-up visit.

There is not absolute consensus about the ideal 25(OH)D level, and as a result we also analyzed 25(OH)D levels continuously [29, 30]. The possible nonlinear relation between serum 25(OH)D and each of these events was also examined nonparametrically with restricted cubic splines [31, 32]. Tests for nonlinearity used the likelihood ratio test, comparing the model with only the linear term to the model with the linear and the cubic spline terms. Sensitivity analyses were conducted by excluding events occurring within 2 months of baseline for all analyses. We also excluded individuals experiencing pulmonary tuberculosis at baseline or during follow-up in anthropometric analyses to investigate the contribution of pulmonary tuberculosis to the associations.

The relationship between 25(OH)D levels and monthly report of clinical signs and symptoms to nurses was analyzed using generalized estimating equations (GEEs) with an exchangeable working covariance and the log link to produce population averaged relative risk estimates [33]. Robust estimators of the variances were used to construct confidence intervals. The robust estimators are consistent estimators of the variances even if the working correlation matrix is misspecified.

Confounders considered for multivariate proportional hazard and GEE models were selected from covariates determined to be risk factors for a 25(OH)D concentration of <30 ng/mL in our previous analysis and include sex, age, season, BMI, WHO HIV disease stage, $CD4^+$ T-cell count, height, and ART regimen at baseline [7]. Effect modification by all covariates listed above, randomized multivitamin regimen, and ART regimen were considered for all analyses. 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 were retained in the analysis, using the missing indicator method for variables. All P values were 2 sided, and a P value of $< .05$ was considered statistically significant. Statistical analyses were performed using the SAS v 9.2 (SAS Institute, Cary, NC).

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

Table 1. Baseline Characteristics of 1103 Human Immunodeficiency Virus (HIV)–Infected Adults Initiating Antiretroviral Therapy (ART) in Dar es Salaam, Tanzania

Characteristic	Value
Female	759 (68.8)
Age, y	
<30	161 (14.6)
30–40	546 (49.5)
40–50	294 (16.7)
>50	102 (9.2)
Season	
Long rain (Dec–Mar)	150 (13.6)
Harvest (Apr–May)	230 (20.9)
Post harvest (Jun–Aug)	637 (57.8)
Short rain (Sept–Nov)	153 (13.9)
BMI[a]	
Severe underweight (<16.0)	73 (6.6)
Underweight (16.0–18.5)	230 (20.9)
Normal (18.5–25) (ref)	637 (57.8)
Overweight (>25.0)	153 (13.9)
WHO HIV disease stage	
I or II	235 (21.3)
III	631 (57.2)
IV	138 (12.5)
$CD4^+$ T-cell count, cells/μL	
<50	214 (19.4)
50–100	197 (17.9)
100–200	429 (38.9)
>200	219 (19.9)
Hemoglobin level, g/L	100.1 ± 22.5
ART regimen prescribed	
d4T, 3TC, NVP	629 (57.0)
d4T, 3TC, EFV	118 (10.7)
AZT, 3TC, NVP	88 (8.0)
AZT, 3TC, EFV	268 (24.3)
Vitamin D level, ng/mL	30.0 ± 8.5

Data are no. (%) of participants or mean ± SD.

Abbreviations: AZT, zidovudine; d4T, stavudine; EFV, efavirenz; NVP, nevirapine; WHO, World Health Organization; 3TC, lamivudine.

[a] Body mass index (BMI) is defined as the weight in kilograms divided by the height in meters squared.

Public Health, Muhimbili University of Health and Allied Sciences, the Tanzania Food and Drug Authority, and the National Institute of Medical Research.

RESULTS

A total of 1103 individuals were randomly selected for 25 (OH)D testing from 3418 individuals enrolled in the parent trial. At baseline, there were no significant differences between the randomly selected cohort and the trial population with respect to age, sex, $CD4^+$ T-cell count, WHO HIV disease stage, or any other variable included in this analysis. Baseline characteristics of the study cohort are presented in Table 1. A total of 101 (9.2%) individuals were classified as vitamin D deficient (25(OH)D level, <20 ng/mL), 481 (43.6%) were characterized as insufficient (25(OH)D level, 20–30 ng/mL), and 521 (47.2%) were characterized as sufficient (25(OH)D level, >30 ng/mL). The median follow-up time for the cohort was 20.6 months (interquartile range, 8.4–33.8).

At monthly clinic visits, study physicians performed a clinical examination and diagnosed, and treated opportunistic infections and other morbidities. Table 2 presents results of proportional hazard models for diagnosis of incident pneumonia, malaria, oral thrush, and pulmonary tuberculosis, by vitamin D status. A total of 43 individuals (4.0%) received a diagnosis of incident pulmonary tuberculosis during follow-up, and all were sputum-smear positive for acid-fast bacilli. After multivariate adjustment, the hazard of incident pulmonary tuberculosis was 2.89 (95% CI, 1.31–7.41; $P = .027$) times greater for individuals with vitamin D deficiency at baseline, compared with vitamin-D-sufficient individuals. There was no significant association between vitamin D insufficiency and incidence of pulmonary tuberculosis (HR, 1.14; 95% CI, .47–1.74; $P = .756$). No linear or nonlinear relationship was detected in a spline analysis of continuous 25(OH)D levels and incident pulmonary tuberculosis. In sensitivity analyses, we excluded pulmonary tuberculosis diagnoses occurring within the first 2 months of follow-up, and categorical results for vitamin D deficiency remained statistically significant (HR, 3.77; 95% CI, 1.43–9.97; $P = .008$). No significant effect modification of the relationship between vitamin D status and the risk of pulmonary tuberculosis was noted by baseline $CD4^+$ T-cell count, age, sex, randomized multivitamin regimen, or ART regimen.

During follow-up, 102 individuals had incident oral thrush diagnosed. After multivariate adjustment, the hazard of oral thrush was 1.96 (95% CI, 1.01–3.81; $P = .046$) times greater for individuals with vitamin D deficiency at baseline, compared with vitamin-D-sufficient individuals. No significant difference for incident oral thrush was found for vitamin-D-insufficient individuals (HR, 1.12; 95% CI, .71–1.76; $P = .632$). There was also no association between vitamin D status and incidence of pneumonia or malaria, in univariate or multivariate analyses. There was no significant effect modification by any variable for oral thrush, pneumonia, or malaria outcomes.

We next examined the relationship of vitamin D status and anthropometric status assessed at each clinic visit (Table 3). Sixty individuals experienced incident wasting (BMI, <18.5) during follow-up. After multivariate adjustment, individuals with vitamin D deficiency had 3.10 times (95% CI, 1.33–7.24; $P = .009$) the hazard of incident wasting as compared to

Table 2. Hazard Ratios (HRs) for Incident Pulmonary Tuberculosis, Pneumonia, Oral Thrush, and Malaria, by Vitamin D Status, Among Human Immunodeficiency Virus (HIV)–Infected Adults Initiating Antiretroviral Therapy (ART) in Dar es Salaam, Tanzania

Outcome (No. of Events)	Vitamin D Sufficient	Vitamin D Insufficient, HR (95% CI)	*P*	Vitamin D Deficient, HR (95% CI)	*P*
Pulmonary Tuberculosis (43)					
Unadjusted	Reference	0.90 (.47–1.74)	.756	2.09 (.88–4.95)	.093
Adjusted[a]	Reference	1.14 (.60–2.18)	.687	2.89 (1.13–7.41)	.027
Pneumonia (325)					
Unadjusted	Reference	0.85 (.67–1.06)	.150	0.85 (.55–1.30)	.453
Adjusted[a]	Reference	0.93 (.73–1.18)	.532	0.79 (.49–1.29)	.344
Oral thrush (102)					
Unadjusted	Reference	0.91 (.60–1.37)	.139	1.59 (.86–2.92)	.139
Adjusted[a]	Reference	1.12 (.71–1.76)	.632	1.96 (1.01–3.81)	.046
Malaria (356)					
Unadjusted	Reference	0.87 (.70–1.09)	.222	0.89 (.60–1.32)	.570
Adjusted[a]	Reference	0.93 (.74–1.18)	.560	1.02 (.66–1.57)	.944

Vitamin D sufficiency was defined as a serum 25-hydroxyvitamin D concentration of ≥30 ng/mL; insufficiency, as a concentration of 20–30 ng/mL; and deficiency, as a concentration of <20 ng/mL. Individuals with outcome events within 1 month of baseline were excluded from analyses.

Abbreviation: CI, confidence interval.

[a] Adjusted for sex and baseline age, season, body mass index (calculated as the weight in kilograms divided by the height in meters squared), World Health Organization HIV disease stage, $CD4^+$ T-cell count, and ART regimen.

vitamin-D-sufficient individuals. There was no significant association between vitamin D insufficiency and wasting, with multivariate adjustment ($P = .433$). No linear or nonlinear relationship was detected in a spline analysis of continuous 25 (OH)D levels and incident wasting. In sensitivity analyses, results for vitamin D deficiency remained significant when we excluded individuals with pulmonary tuberculosis diagnosed at baseline or during follow-up (HR, 2.94; 95% CI, 1.19–7.23;

Table 3. Hazard Ratios for Incident Anemia and Body Mass Index (BMI) Outcomes, by Vitamin D Status, Among Human Immunodeficiency Virus (HIV)–Infected Adults Initiating Antiretroviral Therapy (ART) in Dar es Salaam, Tanzania

Outcome (No. of Events)	Vitamin D Sufficient	Vitamin D Insufficient, HR (95% CI)	*P*	Vitamin D Deficient, HR (95% CI)	*P*
Severe anemia[a] (98)					
Unadjusted	Reference	0.80 (.53–1.21)	.290	0.88 (.42–1.86)	.744
Adjusted[b]	Reference	0.83 (.54–1.27)	.391	0.87 (.41–1.87)	.722
Anemia[a] (66)					
Unadjusted	Reference	0.90 (.54–1.49)	.675	0.79 (.35–1.80)	.573
Adjusted[b]	Reference	0.93 (.54–1.63)	.808	0.76 (.32–1.84)	.547
Wasting, BMI <18.5[c] (60)					
Unadjusted	Reference	1.21 (.70–2.08)	.500	2.26 (1.02–5.03)	.046
Adjusted[b]	Reference	1.27 (.70–2.28)	.433	3.10 (1.33–7.24)	.009
>10% Weight loss[d] (109)					
Unadjusted	Reference	1.20 (.80–1.79)	.042	1.86 (1.02–3.38)	.042
Adjusted[b]	Reference	1.36 (.89–2.061)	.153	2.10 (1.13–3.91)	.019

Vitamin D sufficiency was defined as a serum 25-hydroxyvitamin D concentration of ≥30 ng/mL; insufficiency, as a concentration of 20–30 ng/mL; and deficiency, as a concentration of <20 ng/mL. Individuals with outcome events within 1 month of baseline were excluded from analyses.

Abbreviation: CI, confidence interval.

[a] Severe anemia was defined as a hemoglobin level of <85 g/L for both sexes, and anemia was defined as hemoglobin levels of <120 g/L for women and <130 g/L for men.

[b] Adjusted for sex and baseline age, season, BMI (for anemia and severe anemia outcomes only), World Health Organization HIV disease stage, $CD4^+$ T-cell count, height (for wasting and weight loss outcomes only), and ART regimen.

[c] Calculated as the weight in kilograms divided by the height in meters squared.

[d] Percentage decrease from baseline weight measurement.

$P = .019$), but when we excluded events before 2 months of follow-up, results remained elevated but not statistically significant ($P = .276$).

We also investigated the association of vitamin D with >10% weight loss from the baseline measurement (Table 3). After multivariate adjustment, individuals with vitamin D deficiency had 2.10 times (95% CI, 1.13–3.91; $P = .019$) the hazard of experiencing >10% weight loss as compared to vitamin-D-sufficient individuals. No association was found for vitamin D insufficiency ($P = .153$). No linear or nonlinear relationship was detected in a spline analysis of continuous 25 (OH)D levels and incident >10% weight loss. In sensitivity analyses, weight loss point estimates for vitamin D deficiency remained elevated but were not statistically significant after excluding events before 2 months of follow-up ($P = .132$) and after excluding individuals experiencing pulmonary tuberculosis ($P = .170$). No significant effect modification by baseline BMI, $CD4^+$ T-cell count, age, sex, randomized multivitamin regimen, or ART regimen was found for anthropometric outcomes.

We found no significant association between vitamin D status and incident anemia (hemoglobin level of <120 g/L, for females, and <130 g/L, for males) or severe anemia (hemoglobin level of <85 g/L, for both sexes; Table 3). Similar magnitudes and nonsignificant associations were found when stratifying by sex, and there was no indication of effect modification by any other variable. We also found no significant association between vitamin D deficiency and self-report of any of symptoms to nurses, including cough ($P = .480$), diarrhea ($P = .722$), rash ($P = .224$), neuropathy ($P = .521$), nausea ($P = .994$), fatigue ($P = .904$), and genital discharge/ulcer ($P = .332$).

DISCUSSION

In this study, we found that individuals with deficient levels of vitamin D (25(OH)D level, <20 ng/mL) at ART initiation had significantly increased risk of incident pulmonary tuberculosis and oral thrush as compared to individuals with sufficient levels of vitamin D (25(OH)D level, >30 ng/mL). Individuals with vitamin D deficiency also had increased risk of wasting (BMI, <18.5) and >10% weight loss, with wasting results being robust to exclusion of individuals with pulmonary tuberculosis diagnosed at baseline or during follow-up. There was no association of vitamin D deficiency and incidence of pneumonia, malaria, and self-reported symptoms. We found no association of vitamin D insufficiency (25(OH)D level, 20–30 ng/mL) and incident pulmonary tuberculosis, oral thrush, wasting, or any other outcome.

The study supports results from multiple case-control studies and a single prospective cohort that low vitamin D level is associated with incident active pulmonary tuberculosis [9–16]. There are multiple direct immune mechanisms through which vitamin D deficiency may impair antimycobacterial immunity and increase the risk of tuberculosis reactivation or active primary infection. Vitamin D is required for an interferon-γ-mediated pathway in macrophages that leads to autophagy, phagosomal maturation, and other antimicrobial activities against *M. tuberculosis* [34]. Production of antimicrobial peptides, including cathelicidin and defensin β2, in macrophages when Toll-like receptors are stimulated by 1,25 $(OH)_2D$ [35, 36]. Furthermore, vitamin D also induces reactive nitrogen and oxygen intermediates, suppresses matrix metalloproteinase enzymes, and downregulates tryptophan-aspartate–containing coat protein, which can improve antimycobacterial immune responses [37–39]. Vitamin D may also indirectly reduce the incidence of pulmonary tuberculosis by slowing HIV disease progression. These mechanisms may also explain oral thrush results in this study.

We also found that vitamin D deficiency was significantly associated with incident wasting and >10% weight loss from the baseline measurement. These are important findings in light of strong negative associations between mortality and weight loss after ART initiation [40, 41]. Vitamin D may influence the incidence of wasting or weight loss directly, through immunomodulatory effects, or indirectly, by reducing incidence or severity of comorbid infections that contribute to weight loss. Wasting is characterized by increased production of proinflammatory cytokines, which can inhibit myosin expression and also induce anorexia [42]. Calcitriol (1,25 $(OH)_2D$), the active form of vitamin D, has been shown to reduce production of these proinflammatory cytokines and promote a shift toward an antiinflammatory T-helper 2 response, which may reduce the risk of wasting [5, 6]. Opportunistic and comorbid infections can also contribute to weight loss and wasting in HIV-infected individuals receiving ART [43]. Active pulmonary tuberculosis is associated with wasting and weight loss, but our results were robust to exclusion of individuals experiencing tuberculosis, which may indicate a role of other opportunistic or comorbid infections [44].

In a previous prospective cohort study of pregnant women not receiving ART in Tanzania, low vitamin D level was associated with risk of anemia [45]. In this study, we found no association of vitamin D level and incidence of anemia or severe anemia among the total cohort and when limiting analyses to females. Despite our findings, there is potential for vitamin D level to influence hemoglobin levels through synergistic interaction with erythropoietin and also increased iron storage [46]. Further research is needed to determine whether 25(OH)D level effects the risk of anemia and whether the potential hemoglobin benefits are limited to pregnant women.

The study also has several important limitations. First, we presented an observational cohort study from which reverse causation due to infection cannot be ruled out for our

pulmonary tuberculosis findings, but this risk is minimized, compared with the risk in case-control studies that use active pulmonary tuberculosis cases. Individuals with latent tuberculosis infection may have decreased 25(OH)D levels as compared to individuals with no *M. tuberculosis* infection, and the majority of active pulmonary tuberculosis cases in this study were likely due to activation of latent infections [14]. Randomized controlled trials of vitamin D supplementation are needed to eliminate risk of reverse causation. Second, all pulmonary tuberculosis cases were smear positive, and as a result we likely underestimated the true incidence of tuberculosis, because sputum-smear-negative pulmonary tuberculosis is common in individuals with HIV [47]. As a result, we may have underestimated the association of vitamin D status and tuberculosis incidence if the relationship is the same for sputum-smear-negative tuberculosis cases and misclassification is nondifferential. Third, 25(OH)D level was measured at a single time point, and we are unable to determine whether vitamin D deficiency at a single time point or long-term deficiency is biologically relevant. Last, we may have had limited statistical power to detect effect modification by ART regimen. Multiple studies have shown that antiretrovirals can alter vitamin D metabolism, resulting in decreased level of circulating 25(OH)D, particularly for individuals receiving efavirenz [48, 49].

The observational design of this study precludes determination of a causal effect, and randomized controlled trials of vitamin D supplementation appear to be warranted. Individuals initiating ART may be a particularly suitable population for a vitamin D supplementation and pulmonary tuberculosis trial, because of the high annual risk for reactivation of latent infections, compared with the risk among immunocompetent individuals (5% vs <1%) [50]. Vitamin D supplementation may be a low-cost adjunct treatment to ART if found to be safe and effective in reducing morbidity or mortality.

Notes

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