BMJ open

Vitamin D Status and TB Treatment Outcomes in Adult Tanzanian Patients

Journal:	*BMJ Open*
Manuscript ID:	bmjopen-2013-003703
Article Type:	Research
Date Submitted by the Author:	01-Aug-2013
Complete List of Authors:	Mehta, Saurabh; Cornell University , Mugusi, Ferdinand; Muhimbili University of Health and Allied Sciences, Bosch, Ronald; Harvard University, Aboud, Said; Muhimbili University of Health and Allied Sciences, Urassa, Willy; World Health Organization, Villamor, Eduardo; University of Michigan Ann Arbor, Fawzi, Wafaie; Harvard School of Public Health,
Primary Subject Heading:	Global health
Secondary Subject Heading:	Infectious diseases, HIV/AIDS, Nutrition and metabolism, Epidemiology
Keywords:	Tuberculosis < INFECTIOUS DISEASES, HIV , Africa , Vitamin D

Vitamin D Status and TB Treatment Outcomes in Adult Tanzanian Patients

Saurabh Mehta[1], Ferdinand M. Mugusi[2], Ronald J. Bosch[3], Said Aboud[4], Willy Urassa[5], Eduardo Villamor[6], Wafaie W. Fawzi[7]

Corresponding Author: Saurabh Mehta, M.B.B.S., Sc.D.

Division of Nutritional Sciences, Cornell University

314 Savage Hall, Ithaca NY 14853

Phone: +1-607-255-2640; Fax: +1-607-255-1033; E-mail: smehta@cornell.edu

Word Count: 3738 words

Number of figures: 2

Number of tables: 5

Online Supplementary Information: 2 tables

Running Title: Vitamin D and Tuberculosis

Keywords: Vitamin D, Tuberculosis, HIV, Africa

[1] Division of Nutritional Sciences, Cornell University, Ithaca NY 14853
[2] Department of Internal Medicine, Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania
[3] Department of Biostatistics, Harvard School of Public Health, Boston MA 02115
[4] Department of Microbiology and Immunology, Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania
[5] Diagnostics and Laboratory Technology Team, World Health Organization, Geneva, Switzerland
[6] Department of Epidemiology, University of Michigan School of Public Health, Ann Arbor MI 48109
[7] Departments of Global Health and Population, Nutrition, and Epidemiology, Harvard School of Public Health, Boston MA 02115

Abstract

Objectives: Vitamin D is an immunomodulator and can alter response to tuberculosis treatment, though randomized trials have been inconclusive to date. We present the first comprehensive analysis of the associations between vitamin D status and TB treatment, T-cell counts, and nutritional outcomes by HIV status.

Design: Cohort study

Setting: Outpatient clinics in Dar es Salaam, Tanzania

Participants: 25-hydroxyvitamin D levels were assessed in a cohort of 677 patients with TB (344 HIV-infected) initiating anti-TB treatment at enrollment in a multivitamin supplementation (excluding vitamin D) trial.

Primary and secondary outcome measures: Information on treatment outcomes such as failure and relapse, HIV disease progression, T-cell counts, and anthropometry was collected routinely, with a median follow-up of 52 and 30 months for HIV-uninfected and HIV-infected patients, respectively. Cox and binomial regression, and generalized estimating equations were used to assess the association of vitamin D status with these outcomes.

Results: Mean vitamin D concentrations at enrollment were 69.8 (±21.5) nmol/L [27.9 (±8.6) ng/mL]. Vitamin D insufficiency (<75 nmol/L) was associated with a 66% higher

risk of relapse (95% CI: 4%, 164%; 133% higher risk in HIV-uninfected patients). Each unit higher vitamin D levels at baseline were associated with a decrease of 3 (p=0.004) CD8 and 3 (p=0.01) CD3 T-cells/μL during follow-up in HIV-infected patients. Low vitamin D was also associated with a greater decrease of BMI (-0.21 kg/m^2; 95% CI:-0.39, -0.02), during the first eight months of follow-up. No association was observed for vitamin D status with mortality or HIV disease progression.

Conclusions: Adequate vitamin D status is associated with a lower risk of relapse and with improved nutritional indicators such as BMI in TB patients, with or without HIV infection. Further research is needed to determine the optimal dose of vitamin D and effectiveness of daily vitamin D supplementation among patients with TB.

Article Focus: Recent laboratory data has suggested that optimal vitamin D status may be associated with a more effective immune response to TB infection, a faster rate of bacteriologic cure, and better long-term outcomes. However, clinical and epidemiological studies have found inconsistent results. In this paper, we present the first comprehensive analysis of the associations between vitamin D status and TB treatment, T-cell counts, and nutritional outcomes by HIV status.

Key Messages: We found that patients with adequate vitamin D status were less likely to experience a relapse during follow-up after completing TB treatment. They were also more likely to have a better nutritional status, as assessed by their body mass index, during follow-up, compared to patients with low vitamin D status. The results provide justification for conducting both a dose response study to determine optimal dose of vitamin D and a randomized controlled trial of daily vitamin D supplementation among patients with TB.

Strengths and Limitations of this study: The major strengths of this study include a large number of participants, more than half of whom were HIV-infected, comprehensive assessment of clinical, immunological, socio-demographic, and nutritional parameters, and a long duration of follow-up. On the other hand, the major limitation is the possibility of reverse causation and residual confounding. We have attempted to minimize this through rigorous analyses and adjusting for several potential confounders, including hemoglobin concentrations, HIV status, viral load, CD4 T-cells, and Karnofsky score, in most analyses.

Introduction

Mycobacterium tuberculosis is one of the most pernicious infectious diseases and successful pathogens known to man. More than 95% of the estimated cases and deaths due to tuberculosis (TB) occur in low-income countries. The United Republic of Tanzania is one of the 22 high-burden countries that account for 80% of global TB cases. Tanzania has an incidence of 177 cases per 100,000 population per year and a prevalence of 183 cases per 100,000 population per year [1].

The spread of Human Immunodeficiency Virus (HIV) has fuelled the resurgence of the TB epidemic in Tanzania, as in other parts of sub-Saharan Africa [2]. HIV is the strongest factor in the development of active TB; it is estimated that only one out of ten immunocompetent persons infected with TB develops active TB in his/her lifetime; whereas, one out of ten HIV-infected persons infected with TB will develop active TB every year. An estimated 38% of TB patients in Tanzania are also infected with HIV [1].

Current treatment regimens, given under appropriate management conditions, are nearly 100% curative for patients with drug-susceptible organisms. However, in Tanzania, treatment fails in 12-17% of the cases. Additionally, TB patients in settings such as Tanzania grapple with multiple health-related and quality of life issues, which are not addressed adequately with treatment alone.

Recent data has suggested that optimal vitamin D status may be associated with a more effective immune response to TB infection, a faster rate of bacteriologic cure, and better

long-term outcomes. For example, a recent cross-sectional study found that vitamin D deficiency is highly prevalent in South Africa and is associated with susceptibility to active TB both in the presence and absence of HIV infection [3]. A few randomized trials have also been conducted; two of the recent ones failed to find an effect of vitamin D supplementation on treatment success [4 5]. However, the dose used and duration of supplementation may have precluded finding an effect. Further, most studies had small sample sizes and assessed only a limited number of covariates.

In this manuscript, we comprehensively examined the hypotheses that vitamin D status may be associated with response to treatment, risk of treatment failure, laboratory parameters such as T-cell counts, and anthropometric measurements in the context of a randomized trial of micronutrient supplementation (supplement did not contain vitamin D) in Tanzania to better inform future studies or trials.

Materials and Methods

Study Population: The study population and recruitment methods have been described in detail earlier [6]. Briefly, 887 adults with pulmonary tuberculosis (PTB) were enrolled in a randomized trial to examine the effects of micronutrient supplementation on TB treatment failure, relapse, and mortality. The trial started in April 2000 in Dar es Salaam, Tanzania and continued until April 2005. The eligibility criteria for the study included positive sputum smears for acid-fast bacilli (AFB), age between 18 and 65 years, Karnofsky performance score of ≥ 40% [7], plan to stay in Dar es Salaam for 2 years, not being pregnant, and not having received anti-TB treatment during the previous one year.

Consenting subjects were randomly assigned in computer-generated permuted blocks of 20, stratified by HIV status, to receive a daily oral dose of 1 of 2 regimens: micronutrients (5000 IU of retinol, 20 mg of vitamin B1, 20mg of vitamin B2, 25mg of vitamin B_6, 100 mg of niacin, 50 µg of vitamin B_{12}, 500 mg of vitamin C, 200 mg of vitamin E, 0.8 mg of folic acid, and 100 µg of selenium) or placebo. These doses represent between 6 and 10 times the recommended dietary allowance (RDA) and were being tested at the time among HIV-infected adults from this setting [8]. We chose multiples of the RDA because previous observational studies suggested that HIV-infected individuals need higher dietary intakes of micronutrients to achieve normal serum concentrations [9]. All patients received a daily combination of rifampicin, isoniazid, pyrazinamide, and ethambutol under direct observation of a health worker during the first 2 months (intensive phase) followed by 6 months of self-administered daily isoniazid and ethambutol, as per the Tanzania National TB and Leprosy Programme guidelines. None of the HIV-infected patients received antiretroviral therapy, as these medications were not routinely available in Tanzania at the time this trial was conducted.

At the time of randomization, research nurses collected information on various socio-demographic characteristics including age, education levels, marital status, and socioeconomic status. Anthropometric measurements were also obtained using standardized procedures [10] at the randomization visit as well as during each monthly follow-up visit. Height was measured to the nearest 0.1 cm using SECA Bodymeter 206 stadiometers, weight to the nearest 100 g with SECA 700 balance beam scales, and left

mid-upper arm circumference (MUAC) at the midpoint between the acromion and olecranon to the nearest 0.1 cm using non-stretchable tailor's tapes.

Physician visits were scheduled every 3 months. During these visits, study physicians inquired about the health of the subject during the preceding period and performed a complete physical examination. The stage of HIV disease was assessed according to the World Health Organization system [11].

Ethics Approval: A written informed consent was obtained from all the study participants. The institutional review boards of the Muhimbili University of Health and Allied Sciences, the Tanzanian National AIDS Control Program, and the Harvard School of Public Health approved the study protocol.

Laboratory Methods: At the time of initiation of anti-TB treatment, HIV status was assessed among consenting patients using 2 sequential ELISAs (Wellcozyme, Murex Biotech; Enzygnost anti-HIV1/2 Plus, Dade Behring); discrepant results were resolved by Western Blot test (Bio-rad, Genetic Systems). Both pre-test and post-test counseling was provided. A blood sample also was obtained for measurement of hemoglobin and albumin concentrations using AcT Diff II hematology analyzer (Beckman Coulter, Miami) and Hitachi 911 analyzer (Roche Diagnostics), respectively. CD4, CD3, and CD8 T-cell counts were determined using FACScount or FACSCan systems (Becton Dickinson, CA, USA). Viral load was also determined using the Amplicor HIV-1 monitor v1.5 assay (Roche Molecular Systems, Branchburg, NJ, USA).

Assessment of Vitamin D Status: Serum 25-hydroxyvitamin D concentrations were measured using liquid chromatography-mass spectrometry at the Children's Hospital in Boston only at enrollment. We defined low vitamin D status as serum 25(OH)D levels of less than 75 nmol/L and adequate otherwise. Vitamin D deficiency was defined as serum 25(OH)D levels of less than 50 nmol/L.

Statistical Analysis: We examined the association of vitamin D status with TB treatment outcomes as well as nutritional, immunological, and clinical end points in the entire cohort and separately by HIV status at baseline. TB-related end points included treatment failure, early relapse, and late relapse. Treatment failure by 1 month was defined as positive AFB cultures at 1 month from the initiation of treatment. Relapses were deemed to have occurred in patients with positive cultures, among those who had become culture negative after treatment initiation. Relapses/recurrences included both endogenous reactivation and exogenous reinfection, which could not be distinguished in this study. We calculated the relative risks (RRs) and 95% confidence intervals (CIs) for each of these outcomes by vitamin D status using binomial regression. We used Cox proportional hazards models to assess the association of vitamin D status with mortality in all patients and HIV disease progression from stage 3 to 4 in HIV-infected participants. We defined the end of follow-up as the date when HIV stage was last assessed.

We examined the association of vitamin D status with CD4, CD8, and CD3 T-cell counts, viral load (in HIV-infected participants), indicators of nutritional status (body mass index

[BMI] and albumin concentrations), and hemoglobin concentrations using generalized estimating equations (GEEs). These models do not require that all patients have the same number of follow-up assessments or that the follow-up measurements be obtained at exactly the same time points. We assumed a standard normal distribution for repeated continuous end points (T cell subsets, $\log_{10}$ viral load, anthropometry, and albumin and hemoglobin concentrations) and estimated average differences during follow-up by vitamin D status. We used an exchangeable correlation structure to account for within-subject correlations and adjusted the models for the follow-up time when the measurements had been obtained and for the baseline values.

We analyzed the data for the entire period and for the first 8 months, coinciding with the expected end of TB treatment. Multivariate analyses adjusted for age, Karnofsky score, baseline hemoglobin concentrations, viral load, HIV status, CD4 T-cell counts, and micronutrient supplementation, unless otherwise specified in the results section or the tables. All analyses were performed using SAS software version 9.3 (SAS Institute Inc., Cary NC).

Results

Baseline vitamin D concentrations were available for 677 patients out of the original cohort of 887. Mean 25-hydroxyvitamin D concentration was 69.8 (±21.5) nmol/L [27.9 (±8.6) ng/mL] and its distribution is shown in Figure 1. The baseline characteristics of these 677 patients by HIV status are presented in Table 1. 36% of the HIV-infected patients had CD4 T-cell counts below 200 cells/μL. The mean body mass index (BMI)

was 19.1 ± 2.7 kg/m^2. The median follow-up time for HIV-uninfected patients was 52 months (inter-quartile range [IQR]: 47-57 months) and for HIV-infected patients was 30 months (IQR: 15-41 months).

The mean vitamin D concentrations were significantly different across season of blood draw in this cohort (p=0.004). Tanzania has four seasons: dry (January-February); long rains (March-June); dry (July-October); short rains (November-December). The boxplot of vitamin D's association with season of blood draw is presented in Figure 2. In subgroup analyses, this association was only observed among the HIV-uninfected patients and not the HIV-infected patients.

We examined the correlates of low vitamin D status, defined as serum 25(OH)D concentrations below 75 nmol/L (75 nmol/L) in Supplemental Tables 1 (HIV-uninfected) and 2 (HIV-infected). All factors that had univariate associations with p<0.20 were included in a multivariate model; only the factors that had p<0.05 were retained in the final model. Among the HIV-uninfected subset, patients enrolled in the dry winter season between July and October were 50% more likely to have low vitamin D concentrations, compared to patients enrolled in the dry summer season between January and February (p for season=0.002). Similarly, the participants with the lowest height were more likely to have low vitamin D status (p=0.01). Finally, greater expenditure on food per person per day was associated with a lower risk of having inadequate vitamin D status (Risk Ratio [RR] per 1000 Tanzanian Shillings [approximately 1 US Dollar at the time of the study]: 0.76; 95% Confidence Interval [CI]: 0.59, 0.98). In the HIV-infected subset, patients with

higher hemoglobin concentrations at baseline were less likely to have low vitamin D status, with a 7% lower risk per 1 g/dL higher hemoglobin level (p=0.007). On the other hand, higher number of CD4 T-cells was associated with a higher risk of having inadequate vitamin D status (4% higher risk per 100 CD4 T-cells/μL; p=0.02).

There was no significant association of vitamin D status at TB treatment initiation with mortality or HIV disease progression in this cohort (Table 2). There was no association observed between vitamin D status and treatment failure one month after initiation of TB treatment (Table 3). However, patients with low vitamin D status (<75 nmol/L) had a 66% higher risk of relapse after becoming culture-negative at one month after initiation of TB treatment (95% CI: 4%, 164%). This association was more pronounced in those who were not HIV-infected at enrollment in the study (RR: 2.33; 95% CI: 1.26, 4.29). In analysis with continuous vitamin D levels, each nmol/L increase was associated with a 1% lower risk of relapse during follow-up (p=0.04).

Low vitamin D status was observed to have no association with CD4 T-cell counts during the entire follow-up in either the HIV-infected or the HIV-uninfected subsets (Table 4). However, low vitamin D status was associated with greater CD4 T-cell counts during the first eight months of follow-up in the HIV-infected patients (mean difference: 58; 95% CI: 13, 104).

In analysis among HIV-infected patients with continuous vitamin D levels, each nmol/L higher vitamin D concentration was associated with a decrease of 3 CD8 and 3 CD3 T-

cells per µL. Low vitamin D status was associated with an average of 85 higher CD8 T-cells/µL during follow-up (95% CI: 4, 165). Similar results were observed when we restricted the analyses to the first eight months of follow-up, the duration of TB treatment at the time of the study in Tanzania. No relationship was observed with mean viral loads during follow-up in the patients who were HIV-infected at the time of enrollment.

In analysis examining association of vitamin D status with nutritional parameters in the entire period of follow-up, no significant relationship was observed with BMI, albumin, or hemoglobin concentrations (Table 5). During the first eight months of follow-up, patients with low vitamin D experienced a decline in BMI (Mean: -0.21 kg/m^2; 95% CI: -0.39, -0.02), compared to patients with adequate vitamin D status. These results were more pronounced in HIV-uninfected patients (Mean: -0.34; 95% CI: -0.60, -0.09) and not significant in HIV-infected patients. HIV-infected patients with low vitamin D status had increased albumin levels (Mean: 0.94; 95% CI: 0.55, 1.32) during the first eight months of follow-up compared to patients with adequate vitamin D status.

Discussion

In this study among 677 patients with tuberculosis in Tanzania, more than 61% of the participants had vitamin D concentrations below 75 nmol/L (75 nmol/L). Vitamin D concentrations were associated with the season of blood draw, money spent on food per person per day, and height in HIV-uninfected participants and hemoglobin concentrations and CD4 T-cell counts among HIV-infected patients. Low vitamin D status (<75 nmol/L) was not associated with mortality, HIV disease progression, or treatment failure during

follow-up in the entire cohort. However, patients with low vitamin D status had an increased risk of experiencing TB relapse during follow-up. Further, low vitamin D status was associated with a decline in CD8 and CD3 T-cells in both the first eight months (the duration of TB treatment) and the entire period of follow-up. A similar relationship was observed with BMI in the first eight months of follow-up.

Our study was conducted in Dar es Salaam, the largest urban center in Tanzania, and just six degrees south of the Equator. The prevalence of low vitamin D status (>61%) in this study is higher than the approximately 40% found in a previous study among TB patients in Mwanza, Tanzania [12] and in our studies among HIV-infected pregnant women (~85% of them had stage 1 HIV disease, unlike this study) in Dar es Salaam [13 14]. However, this prevalence is lower than what was observed in a cross-sectional study in South Africa, where 88% of HIV-uninfected and 97% of HIV-infected TB patients had low vitamin D status. The mean vitamin D concentration in this study was 69.8 nmol/L, compared to 86.5 nmol/L in the study in Mwanza and 28.8-40 nmol/L in the South African study. One study from Thailand observed similar levels (69.0 nmol/L) in TB patients [15]; Thailand is located at a similar distance from the Equator as Tanzania, though it is in the northern hemisphere. Similar to the study in South Africa, the vitamin D levels were lowest in our study in the dry winter season between July and October, though the differences were not as stark. For example, the mean vitamin D concentration in January to March in the South African study was 56.8 nmol/L and 30.8 nmol/L between July and September, whereas in our study, the concentrations were 74.8 nmol/L for January through February, and 66.3 nmol/L for July through October.

Vitamin D is synthesized in the skin through the action of ultraviolet light on 7-dehydrocholesterol. Fatty fish, such as salmon and sardines, are good sources of vitamin D in the diet but are not widely available everywhere and are usually expensive. Increasing urbanization and a tendency to spend most time indoors are major factors that contribute to the inability of the skin to synthesize adequate amounts of vitamin D [16-18]. Additionally, the TB disease itself and/or the HIV co-infection in the participants in this study are probably the primary reasons for restricted physical activity, lack of adequate exposure to sunlight, and consequent low concentrations of vitamin D.

Several other investigators have examined correlates of vitamin D status in TB patients. The study in Mwanza found that marital status, BMI, and serum transferrin receptor concentrations were correlated with vitamin D status. Though the first two were correlated with vitamin D status in our study in univariate analyses, neither remained significant in multivariate analyses. We didn't measure serum transferrin receptor in our study, though we did observe a correlation of vitamin D status with hemoglobin concentrations among the HIV-infected subset. Another study in South Africa found that TB status (active disease *vs.* latent infection), month of sampling, and BMI were significantly correlated with vitamin D status in multivariate analyses [3]. All patients in our study had active disease, and we didn't observe a relationship with BMI in our analyses. The study in South Africa incorporated only those correlates associated with serum 25(OH)D concentration with $P < 0.05$ in univariate analysis in the multivariate model. This may have precluded selection of important covariates and confounders, if

measured, and produced biased estimates and confidence intervals; increasing the nominal significance level to 20% or more, as used in this study [19] can eliminate most of this bias. Most other studies have been with smaller sample sizes and have examined a limited set of covariates, compared to the current study.

There was no association of vitamin D status with mortality or HIV disease progression in this cohort, unlike our previous studies among HIV-infected pregnant women [13 20] or HIV-infected adults [21 22] in Tanzania. The major difference is that in our earlier studies [13 20], a large majority (~85%) of the participants had stage 1 or asymptomatic HIV disease, whereas in this study, most of the individuals were already at stage 3 disease.

The association of low vitamin D status with TB recurrence/relapse, primarily driven by the HIV-uninfected subset, is a novel finding in a longitudinal study and has important implications. Vitamin D deficiency has been linked to TB in several studies – a hypothesis perhaps initially generated by the observed seasonality of TB. *In vitro* and animal studies indicate that 1,25-dihydroxyvitamin D_3, the most active form of vitamin D, may increase mycobacterial killing by macrophages but also limits host damage by decreasing the gamma-interferon production [23-28]. In perhaps the strongest evidence to date for a role of vitamin D in tuberculosis, a study by Liu *et al* [29] found that the antimycobacterial response in humans is dependent on adequate availability of vitamin D.

A few randomized trials of vitamin D supplementation in TB patients have been conducted in the past few years [4 5 30]. In a randomized trial that was conducted among 365 TB patients in Guinea-Bissau starting antituberculosis treatment, overall mortality was 15% (54 of 365) at 1 year of follow-up and similar in both arms [5]. Martineau and colleagues didn't find a difference in median time to sputum culture conversion with vitamin D supplementation of 2.5 mg vitamin D3 at enrollment, 14, 28, and 42 days after starting TB treatment in 126 adults with sputum smear-positive PTB [4]. A recent report by Coussens *et al* from a subset of the 126 adults included in the trial above stated that median time to sputum smear conversion in the intervention arm was significantly shorter than in the control arm (23 *vs.* 36 days; p=0.04) [30]. The lack of effect and concordance in most of these trials is probably due to the dose and dosing interval used. It is worth noting however, that large intermittent doses of vitamin D may result in supraphysiological concentrations in some cases, which may be more harmful than helpful in their effects on the immune system [31].

Low vitamin D status also was associated with T-cell subset counts only among the HIV-infected patients in this cohort. We can only speculate as to the reasons for the significantly higher increase in CD4 T-cells observed in patients with low vitamin D levels at baseline. One potential explanation is that HIV-infected patients with low vitamin D status may experience more uncontrolled immune reconstitution, leading to a greater increase in CD4 T-cell counts, on treatment of TB, compared to patients with adequate vitamin D status. This may also explain why this relationship is observed only in the first eight months of follow-up and not subsequently.

The results for CD8 and CD3 T-cells are consistent with our previous studies among HIV-infected women in Tanzania [13 14]. This could suggest a possible role of vitamin D in inflammation. Although, the conventional role of CD8 cells is as cytotoxic cells, they also are effector cells in inflammation [32]. The involvement of vitamin D in modulating CD8 cells is also indicated by the fact that CD8 cells express the highest concentration of vitamin D receptor of the immune cells [33]. Other studies also have found that vitamin D suppresses antigen-stimulated proinflammatory cytokine responses, which may help speed up resolution of inflammatory responses that can lead to increased risk of mortality among TB patients [30].

TB, once known as 'consumption', is associated with significant wasting and weight loss. The observation that better vitamin D status among HIV-uninfected patients is associated with a greater increase in BMI during follow-up is likely related to decreased risk of relapse among these patients, as well as improvement in quality of life through mechanisms such as better metabolism that were not directly assessed in this study.

The major strengths of this study include a large number of participants, more than half of whom were HIV-infected, comprehensive assessment of clinical, immunological, socio-demographic, and nutritional parameters, and a long duration of follow-up. On the other hand, the major limitation is the possibility of reverse causation and residual confounding. We have attempted to minimize this through rigorous analyses and adjusting for several potential confounders, including hemoglobin concentrations, HIV status, viral load, CD4 T-cells, and Karnofsky score, in most analyses. The study results

are generalizable to most settings with a high TB burden and widely prevalent vitamin D insufficiency.

In summary, our study results indicate that adequate vitamin D status is associated with better clinical and nutritional parameters during follow-up in a cohort of TB patients in Tanzania. While randomized trials of vitamin D supplementation among TB patients are urgently warranted, it is also imperative to conduct dose-response studies to determine ideal dose and duration for the supplement.

Acknowledgments

We thank the patients, whose participation made this study possible. We are grateful to the study coordinator, Dorothy Mallya, and to the study physicians, research nurses, and laboratory and administrative staff at Muhimbili University of Health and Allied Sciences in Dar es Salaam, Tanzania, for their contributions to the project.

Financial support

National Institute of Allergy and Infectious Diseases, National Institutes of Health (grant U01AI045441 to support the study)

The authors report NO conflict of interest

Author Contributions

SM wrote the first draft of the manuscript and analyzed and interpreted the data; FMM, RJB, SA, WU, EV, and WWF were investigators of the parent trial and contributed to field activities and oversight; RJB also helped with the analysis and interpretation of the data; all authors participated in study design and contributed to the final manuscript. All authors have also read and approved the final manuscript.

References

1. WHO. Global Tuberculosis Control. Geneva: World Health Organization, 2011.

2. Chaisson RE, Martinson NA. Tuberculosis in Africa--combating an HIV-driven crisis. N Engl J Med 2008;**358**(11):1089-92 doi: 358/11/1089 [pii] 10.1056/NEJMp0800809[published Online First: Epub Date]|.

3. Martineau AR, Nhamoyebonde S, Oni T, et al. Reciprocal seasonal variation in vitamin D status and tuberculosis notifications in Cape Town, South Africa. Proc Natl Acad Sci U S A 2011;**108**(47):19013-7 doi: 10.1073/pnas.1111825108[published Online First: Epub Date]|.

4. Martineau AR, Timms PM, Bothamley GH, et al. High-dose vitamin D(3) during intensive-phase antimicrobial treatment of pulmonary tuberculosis: a double-blind randomised controlled trial. Lancet 2011;**377**(9761):242-50 doi: 10.1016/S0140-6736(10)61889-2[published Online First: Epub Date]|.

5. Wejse C, Gomes VF, Rabna P, et al. Vitamin D as supplementary treatment for tuberculosis: a double-blind, randomized, placebo-controlled trial. American Journal of Respiratory and Critical Care Medicine 2009;**179**(9):843-50 doi: 10.1164/rccm.200804-567OC[published Online First: Epub Date]|.

6. Villamor E, Mugusi F, Urassa W, et al. A Trial of the Effect of Micronutrient Supplementation on Treatment Outcome, T Cell Counts, Morbidity, and Mortality in Adults with Pulmonary Tuberculosis. J Infect Dis 2008;**197**(11):1499-505 doi: 10.1086/587846 10.1086/587846 [pii][published Online First: Epub Date]|.

7. Karnofsky DA, Abelmann WH, Craver LF, et al. The use of nitrogen mustards in the palliative treatment of cancer. Cancer 1948;**1**:634-56

8. Fawzi WW, Msamanga GI, Spiegelman D, et al. A randomized trial of multivitamin supplements and HIV disease progression and mortality. N Engl J Med 2004;**351**(1):23-32

9. Baum M, Cassetti L, Bonvehi P, et al. Inadequate dietary intake and altered nutrition status in early HIV-1 infection. Nutrition 1994;**10**(1):16-20

10. Lohman TG, Roche AF, Martorell R. *Anthropometric standardization reference manual*. Champaign, IL: Human Kinetics Books, 1988.

11. WHO. Interim proposal for a WHO staging system for HIV infection and disease. Wkly Epidemiol Rec 1990;**65**:221-24

12. Friis H, Range N, Pedersen ML, et al. Hypovitaminosis D is common among pulmonary tuberculosis patients in Tanzania but is not explained by the acute phase response. J Nutr 2008;**138**(12):2474-80 doi: 138/12/2474 [pii] 10.3945/jn.108.094979[published Online First: Epub Date]|.

13. Mehta S, Giovannucci E, Mugusi FM, et al. Vitamin D status of HIV-infected women and its association with HIV disease progression, anemia, and mortality. PLoS ONE 2010;**5**(1):e8770 doi: 10.1371/journal.pone.0008770[published Online First: Epub Date]|.

14. Mehta S, Spiegelman D, Aboud S, et al. Lipid-soluble vitamins A, D, and E in HIV-infected pregnant women in Tanzania. European journal of clinical nutrition 2010;**64**(8):808-17 doi: 10.1038/ejcn.2010.76[published Online First: Epub Date]|.

15. Davies PD, Church HA, Bovornkitti S, et al. Altered vitamin D homeostasis in tuberculosis. Intern Med (Thailand) 1988;**4**:45-47

16. Norman AW. Nutritional Aspects of Vitamin D. http://vitamind.ucr.edu/nutri.html. Accessed Oct 9, 2006. 1999. http://vitamind.ucr.edu/nutri.html (accessed Oct 9, 2006).

17. Utiger RD. The need for more vitamin D. N Engl J Med 1998;**338**(12):828-9

18. Holick MF. High prevalence of vitamin D inadequacy and implications for health. Mayo Clin Proc 2006;**81**(3):353-73

19. Greenland S. Modeling and variable selection in epidemiologic analysis. Am J Public Health 1989;**79**(3):340-9

20. Mehta S, Mugusi FM, Spiegelman D, et al. Vitamin D status and its association with morbidity including wasting and opportunistic illnesses in HIV-infected women in Tanzania. AIDS Patient Care STDS 2011;**25**(10):579-85 doi: 10.1089/apc.2011.0182[published Online First: Epub Date]|.

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. The Journal of infectious diseases 2013;**207**(3):378-85 doi: 10.1093/infdis/jis693[published Online First: Epub Date]|.

22. Sudfeld CR, Wang M, Aboud S, et al. Vitamin D and HIV progression among Tanzanian adults initiating antiretroviral therapy. PloS one 2012;**7**(6):e40036 doi: 10.1371/journal.pone.0040036[published Online First: Epub Date]|.

23. Thoma-Uszynski S, Stenger S, Takeuchi O, et al. Induction of direct antimicrobial activity through mammalian toll-like receptors. Science 2001;**291**(5508):1544-7

24. Waters WR, Palmer MV, Nonnecke BJ, et al. Mycobacterium bovis infection of vitamin D-deficient NOS2-/- mice. Microb Pathog 2004;**36**(1):11-7

25. Waters WR, Nonnecke BJ, Foote MR, et al. Mycobacterium bovis bacille Calmette-Guerin vaccination of cattle: activation of bovine CD4+ and gamma delta TCR+ cells and modulation by 1,25-dihydroxyvitamin D3. Tuberculosis (Edinb) 2003;**83**(5):287-97

26. Waters WR, Nonnecke BJ, Rahner TE, et al. Modulation of Mycobacterium bovis-specific responses of bovine peripheral blood mononuclear cells by 1,25-dihydroxyvitamin D(3). Clin Diagn Lab Immunol 2001;**8**(6):1204-12

27. Kawakami K, Teruya K, Tohyama M, et al. [A therapeutic trial of experimental tuberculosis with gamma-interferon in an immunocompromised mouse model]. Kekkaku 1994;**69**(10):607-13

28. McMurray DN, Bartow RA, Mintzer CL, et al. Micronutrient status and immune function in tuberculosis. Ann N Y Acad Sci 1990;**587**:59-69

29. Liu PT, Stenger S, Li H, et al. Toll-Like Receptor Triggering of a Vitamin D-Mediated Human Antimicrobial Response. Science 2006:1123933 doi: 10.1126/science.1123933[published Online First: Epub Date]|.

30. Coussens AK, Wilkinson RJ, Hanifa Y, et al. Vitamin D accelerates resolution of inflammatory responses during tuberculosis treatment. Proc Natl Acad Sci 2012:Epub

31. Martineau AR. Bolus-dose vitamin D and prevention of childhood pneumonia. Lancet 2012;**379**(9824):1373-5 doi: 10.1016/S0140-6736(12)60405-X[published Online First: Epub Date]|.

32. Babbe H, Roers A, Waisman A, et al. Clonal expansions of CD8(+) T cells dominate the T cell infiltrate in active multiple sclerosis lesions as shown by micromanipulation and single cell polymerase chain reaction. J Exp Med 2000;**192**(3):393-404

33. Veldman CM, Cantorna MT, DeLuca HF. Expression of 1,25-dihydroxyvitamin D(3) receptor in the immune system. Arch Biochem Biophys 2000;**374**(2):334-8 doi: 10.1006/abbi.1999.1605 S0003-9861(99)91605-3 [pii][published Online First: Epub Date]|.

Figure Legends

1. Distribution of Vitamin D concentrations at baseline (nmol/L)

2. Distribution of Vitamin D concentrations by season of blood draw; Season 1: Dry (January-February); Season 2: Long Rains (March-June); Season 3: Dry (July-October); Season 4: Short Rains (November-December)

Tables

Table 1	**Baseline Characteristics of the Study Population (n=677)**		
		HIV-infected (n=344)	**HIV-uninfected (n=333)**
Variable		**Mean (Standard Deviation)**	**Mean (Standard Deviation)**
Age, years		34.4 ± 8.6	30.2 ± 9.2
Money spent on food per person per day, Tanzanian Shillings*		587.3 ± 445.9	580.1 ± 684.2
Hemoglobin, g/dL		9.9 ± 1.8	11.1 ± 1.7
Albumin, g/dL		2.8 ± 1.0	3.2 ± 1.1
CD3 T-cell count, cells/μL		1228.0 ± 608.5	1195.9 ± 404.8
CD4 T-cell counts, cells/μL		327.2 ± 246.2	709.2 ± 250.8
CD8 T-cell counts, cells/μL		826.9 ± 447.5	427.5 ± 188.2
Log(10) Viral Load, copies/mL		4.6 ± 1.0	N/A
Body Mass Index, kg/m^2		19.4 ± 2.8	18.8 ± 2.5
Mid-Upper Arm Circumference, cm		23.4 ± 2.7	23.1 ± 2.7
Follow-up time, days		916.8 ± 507.4	1532.9 ± 331.4
		n (%)	***n (%)***
Low Vitamin D (serum 25-hydroxyvitamin D <75 nmol/L)		218 (63.4%)	200 (60.1%)

Vitamin D deficiency (serum 25-hydroxyvitamin D <50 nmol/L)		55 (16.0%)	51 (15.3%)
Sex			
	Male	203 (59.0%)	257 (77.2%)
	Female	141 (41.0%)	76 (22.8%)
Center			
	Mwananyamala	79 (23.0%)	88 (26.4%)
	Temeke	102 (29.7%)	83 (24.9%)
	Tandale	83 (24.1%)	91 (27.3%)
	Mbgala	31 (9.0%)	70 (21.0%)
	Amana	49 (14.2%)	1 (.3%)
Karnofsky Score <70%		45 (13.1%)	29 (8.7%)
Education Group			
	None	29 (8.4%)	36 (10.8%)
	Low <5years	35 (10.2%)	31 (9.3%)
	Primary 5-8 years	238 (69.2%)	233 (70.0%)
	Secondary/University	42 (12.2%)	33 (9.9%)

Cohabits with a Partner		200 (58.1%)	168 (50.5%)
Assets at home			
	None	92 (26.9%)	108 (32.4%)
	One	89 (26.0%)	85 (25.5%)
	2-3	122 (35.7%)	114 (34.2%)
	4-5	39 (11.4%)	26 (7.8%)
WHO HIV Disease Stage			
	3	240 (90.9%)	N/A
	4	24 (9.1%)	
CD4 T-cell categories, cells/μL			
	0-199	97 (35.9%)	0 (.0%)
	200-499	116 (43.0%)	69 (22.9%)
	500+	57 (21.1%)	232 (77.1%)
WHO BMI Group, kg/m^2			
	<16	26 (7.7%)	33 (9.9%)
	16-16.99	37 (10.9%)	45 (13.6%)
	17-18.49	73 (21.5%)	88 (26.5%)
	18.5-19.99	79 (23.3%)	70 (21.1%)
	20-21.99	77 (22.7%)	69 (20.8%)

	22+	47 (13.9%)	27 (8.1%)

*1 US Dollar ≅ 1000 Tanzanian Shillings at the time of the study

Table 2	Vitamin D Status and Mortality and HIV Disease Progression in HIV-infected TB Patients				
		Univariate		Multivariate	
Outcome	*n/N (%)*	*RR (95% CI)*	*p-value*	*RR (95% CI)*	*p-value*
Mortality					
Low Vitamin D (<75 nmol/L)	61/218 (28.0%)	0.73 (0.50, 1.08)	0.12	0.70 (0.47, 1.04)	0.08
Adequate Vitamin D	43/126 (34.1%)				
Vitamin D deficient (<50 nmol/L)	20/55 (36.4%)	1.34 (0.82, 2.18)	0.25	0.91 (0.55 1.50)	0.71
Not deficient	84/289 (29.1%)				
Continuous Vitamin D (nmol/L)		1.00 (0.99, 1.01)	0.49	1.01 (1.00, 1.02)	0.15
HIV Disease Progression					
Low Vitamin D	46/150	1.10 (0.67, 1.82)	0.71	1.08 (0.64, 1.82)	0.78

	(<75 nmol/L)	(30.7%)					
	Adequate Vitamin D	23/90 (25.6%)		Reference		Reference	
	Vitamin D deficient (<50 nmol/L)	14/34 (41.2%)		1.91 (1.05, 3.44)	**0.03**	1.48 (0.78, 2.82)	0.23
	Not deficient	55/206 (26.7%)		Reference		Reference	
	Continuous Vitamin D (nmol/L)			0.99 (0.98, 1.01)	0.30	1. 00 (0.99, 1.01)	0.57

p-values obtained using Cox Proportional Hazards Regression; RR: Risk Ratio; 95% CI: 95% Confidence Interval

Multivariate analyses adjusted for Age, Karnofsky Score, Baseline Hemoglobin, Viral Load, HIV Status, CD4 Counts, and Micronutrient Supplementation

Table 3	Vitamin D Status and Treatment Outcomes in TB Patients					
		Univariate		Multivariate		
Outcome	*n/N (%)*	*RR (95% CI)*	*p-value*	*RR (95% CI)*	*p-value*	
Treatment Failure by 1 month post-treatment initiation						
Low Vitamin D (<75 nmol/L)	58/298 (19.5%)	1.06 (0.72, 1.55)	0.77	1.02 (0.70, 1.49)	0.93	
Adequate Vitamin D	34/185 (18.4%)					
Vitamin D deficient (<50 nmol/L)	15/75 (20.0%)	1.06 (0.65, 1.74)	0.82	1.13 (0.69, 1.86)	0.63	
Not deficient	77/408 (18.9%)					
Continuous Vitamin D (nmol/L)		1.00 (0.99, 1.01)	0.49	1. 00 (0.99, 1.01)	0.50	
Any Relapse (relapse after 1 month post-treatment initiation if culture negative at 1 month)						
Low Vitamin D (<75	51/227 (22.5%)	1.56 (0.98, 2.48)	0.06	1.66 (1.04, 2.64)	**0.03**	

	nmol/L)							
	Adequate Vitamin D	21/146 (14.4%)						
	Vitamin D deficient (<50 nmol/L)	13/56 (23.2%)		1.25 (0.73, 2.12)	0.41		1.40 (0.82, 2.39)	0.21
	Not deficient	59/317 (18.6%)						
	Continuous Vitamin D (nmol/L)			0.99 (0.98, 1.00)	0.06		0.99 (0.98, 1.00)	**0.04**

p-values obtained using Binomial Regression; RR: Risk Ratio; 95% CI: 95% Confidence Interval

Multivariate analyses adjusted for Age, Karnofsky Score, Baseline Hemoglobin, Viral Load, HIV Status, CD4 Counts, and Micronutrient Supplementation

Table 4 Vitamin D Status and T-cell Counts (cells/µL) in TB Patients

Outcome		CD4 T-cells				CD8 T-cells				CD3 T-cells			
		Adequate vitamin D, mean (SD)[a]	*Low vitamin D, mean difference (95% CI)*[b]	*Low vitamin D, adjusted mean difference (95% CI)*[c]	*p-value*	*Adequate vitamin D, mean (SD)*[a]	*Low vitamin D, mean difference (95% CI)*[b]	*Low vitamin D, adjusted mean difference (95% CI)*[c]	*p-value*	*Adequate vitamin D, mean (SD)*[a]	*Low vitamin D, mean difference (95% CI)*[b]	*Low vitamin D, adjusted mean difference (95% CI)*[c]	*p-value*
Entire follow-up: HIV-infected patients													

	Low Vitamin D (<75 nmol/L)		300 (234)	17 (-23, 56)	21 (-18, 59)	0.29		902 (457)	88 (7, 169)	85 (4, 165)	**0.04**		1298 (635)	101 (-4, 206)	103 (-5, 212)	0.06
	Vitamin D deficient (<50 nmol/L)		333 (225)	21 (-34, 76)	30 (-26, 86)	0.29		957 (424)	105 (-9, 219)	114 (-6, 234)	0.06		1392 (595)	104 (-47, 255)	125 (-28, 279)	0.11
	Continuous Vitamin D (per nmol/L)			0 (-1, 1)	-1 (-1, 0)	0.26			-3 (-5, -1)	-3 (-5, -1)	**0.004**			-3 (-5, -1)	-3 (-6, -1)	**0.01**
Entire follow-up: HIV-uninfected patients																
	Low Vitamin D (<75 nmol/L)		771 (235	-2 (-49,	3 (-45, 51)	0.91		508 (209	-25 (-63, 14)	-22 (-60, 17)	0.27		1351 (400)	-37 (-109,	-28 (-99, 44)	0.45

			)	45)				)						35)		
	Vitamin D deficient (<50 nmol/L)		781 (241)	-34 (-99, 30)	-34 (-101, 32)	0.31		500 (195)	-1 (-64, 62)	3 (-61, 67)	0.93		1354 (397)	-33 (-136, 71)	-28 (-134, 79)	0.61
	Continuous Vitamin D (per nmol/L)			0 (-1, 1)	0 (-1, 1)	0.97			0 (-1, 1)	0 (-1, 1)	0.83			0 (-1, 2)	0 (-2, 2)	0.90
First 8 months of follow-up: HIV-infected patients																
	Low Vitamin D (<75 nmol/L)		316 (237)	54 (8, 100)	58 (13, 104)	**0.01**		868 (470)	132 (29, 235)	119 (15, 223)	**0.02**		1279 (670)	190 (42, 337)	179 (28, 331)	**0.02**

	Vitamin D deficient (<50 nmol/L)		372 (264)	36 (-25, 97)	41 (-20, 101)	0.19		963 (471)	63 (-77, 203)	75 (-72, 221)	0.32		1443 (689)	67 (-125, 259)	101 (-93, 295)	0.31
	Continuous Vitamin D (per nmol/L)			-1 (-2, 0)	-1 (-2, 0)	**0.01**			-4 (-6, -1)	-4 (-6, -1)	**0.002**			-4 (-7, -1)	-5 (-8, -2)	**0.003**
First 8 months of follow-up: HIV-uninfected patients																
	Low Vitamin D (<75 nmol/L)		724 (243)	1 (-52, 53)	6 (-47, 59)	0.82		461 (232)	-22 (-63, 20)	-17 (-57, 22)	0.39		1248 (446)	-38 (-121, 46)	-27 (-106, 52)	0.50
	Vitamin D deficient (<50		731 (237	-7 (-95,	-7 (-96, 81)	0.87		454 (209	4 (-73, 81)	5 (-71, 81)	0.90		1247 (410)	14 (-124,	17 (-123,	0.82

	nmol/L)		)	80)				)						153)	156)	
	Continuous Vitamin D (per nmol/L)			0 (-2,1)	0 (-2,1)	0.54			0 (-1, 1)	0 (-1, 1)	0.73			0 (-2, 1)	0 (-2, 1)	0.57

[a] Data are the means (SD) of the average measurement during follow-up for each participant

[b] Data are the mean difference between the low and the adequate vitamin D group, as defined in Column B. The mean differences, 95% confidence intervals (CIs), and corresponding p-values were estimated from generalized estimating equations, after adjustment for baseline measurements, follow-up time, and treatment (micronutrients *vs.* placebo) group.

[c] Multivariate analyses additionally adjusted for Age, Karnofsky Score, and Baseline Hemoglobin

Table 5	Vitamin D Status and Nutritional Parameters in TB Patients													
	Body Mass Index (kg/m^2)					Albumin concentration (g/dL)					Hemoglobin concentration (g/dL)			
Outcome	*Adequate vitamin D, mean (SD)[a]*	*Low vitamin D, mean difference (95% CI)[b]*	*Low vitamin D, adjusted mean difference (95% CI)[c]*	*p-value*		*Adequate vitamin D, mean (SD)[a]*	*Low vitamin D, mean difference (95% CI)[b]*	*Low vitamin D, adjusted mean difference (95% CI)[c]*	*p-value*		*Adequate vitamin D, mean (SD)[a]*	*Low vitamin D, mean difference (95% CI)[b]*	*Low vitamin D, adjusted mean difference (95% CI)[c]*	*p-value*
Entire follow-up:														

All patients														
Low Vitamin D (<75 nmol/L)	21.20 (2.80)	-0.06 (-0.30, 0.17)	-0.08 (-0.30, 0.14)	0.46		3.42 (0.74)	-0.05 (-0.14, 0.04)	0.00 (-0.08, 0.08)	0.97		12.65 (1.80)	-0.16 (-0.40, 0.08)	-0.18 (-0.41, 0.05)	0.12
Vitamin D deficient (<50 nmol/L)	21.23 (3.00)	-0.16 (-0.46, 0.14)	-0.14 (-0.44, 0.15)	0.34		3.42 (0.72)	-0.05 (-0.17, 0.07)	0.02 (-0.08, 0.13)	0.65		12.42 (1.87)	0.15 (-0.16, 0.45)	0.17 (-0.11, 0.45)	0.24
Continuous Vitamin D (per nmol/L)		0.00 (0.00, 0.01)	0.00 (0.00,	0.30			0.002 (0.00,	0.000 (-	0.90			0.00 (-0.01,	0.00 (0.00,	0.85

			0.01)				0.004)	0.002, 0.002)				0.01)	0.01)	
First 8 months of follow-up: All patients														
Low Vitamin D (<75 nmol/L)	20.96 (2.73)	-0.20 (-0.40, -0.01)	-0.21 (-0.39, -0.02)	**0.03**	3.42 (1.09)	-0.01 (-0.18, 0.16)	0.04 (-0.13, 0.21)	0.65	12.12 (1.85)	-0.01 (-0.28, 0.26)	-0.04 (-0.31, 0.23)	0.78
Vitamin D	20.85 (2.84)	0.00 (-0.25, 0.25)	0.04 (-0.21,	0.78	3.41 (1.08)	-0.11 (-0.32,	-0.05 (-0.27,	0.64	11.92 (1.99)	0.16 (-0.18,	0.21 (-0.10,	0.19

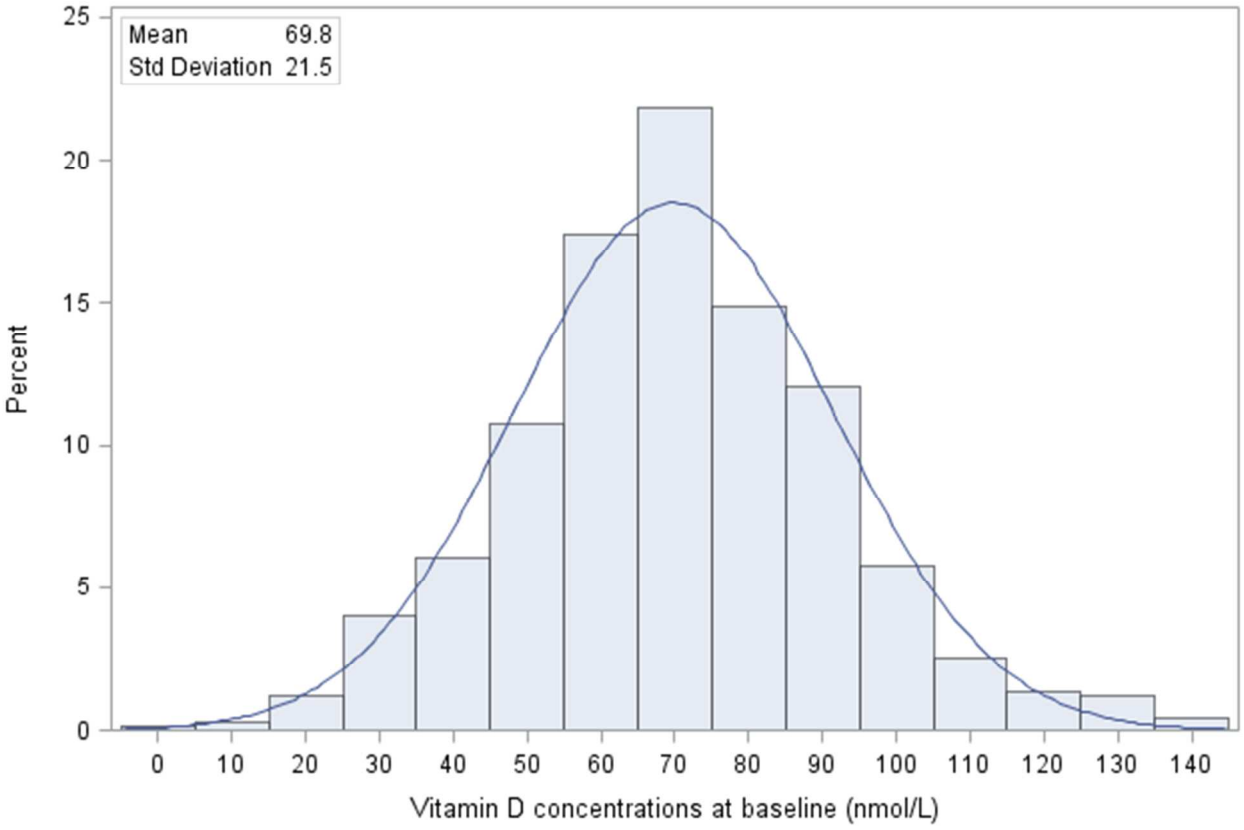
	deficient (<50 nmol/L)			0.29)				0.10)	0.17)				0.50)	0.53)	
	Continuous Vitamin D (per nmol/L)	0.00 (0.00, 0.01)		0.00 (0.00, 0.01)	0.38			0.000 (-0.003, 0.005)	0.000 (-0.004, 0.004)	0.87			0.00 (-0.01, 0.00)	0.00 (-0.01, 0.00)	0.57

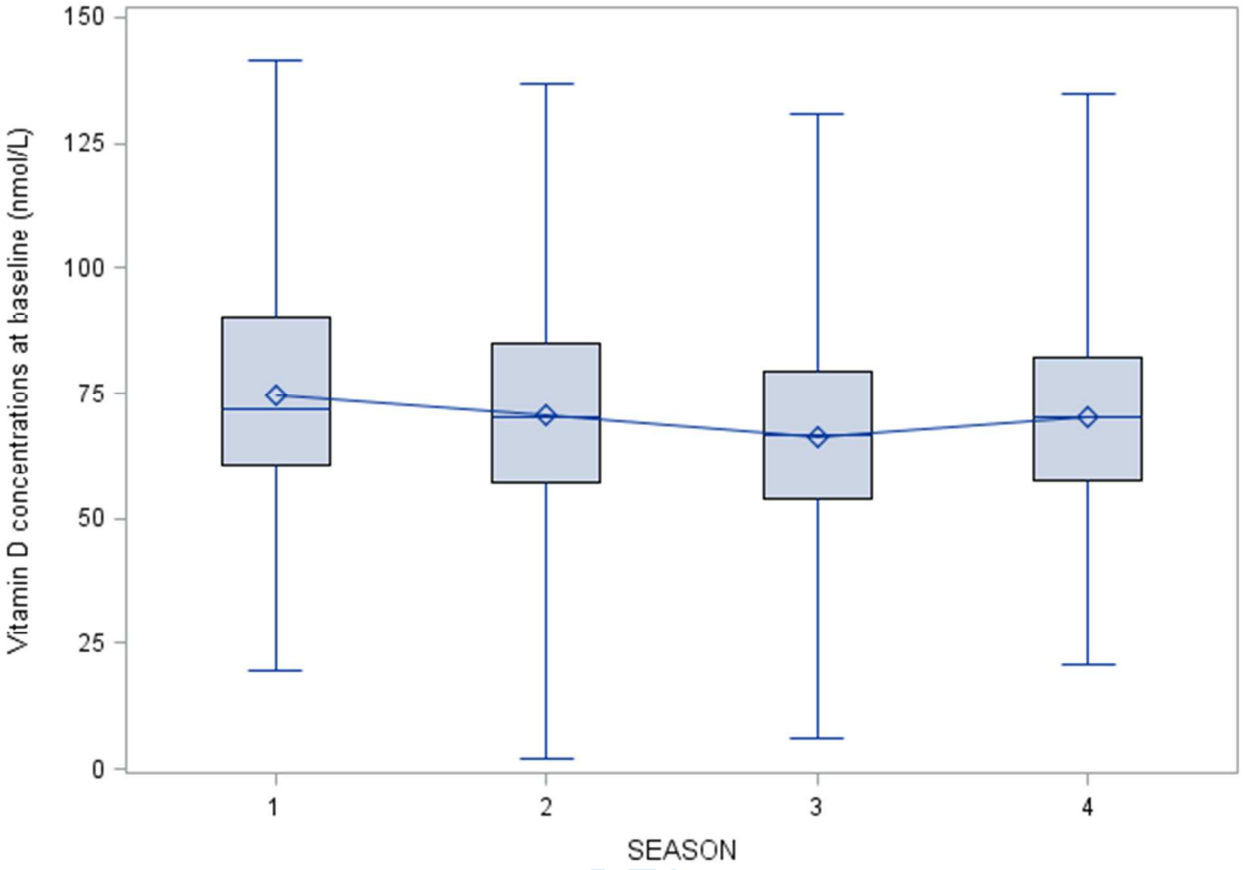
[a] Data are the means (SD) of the average measurement during follow-up for each participant

[b] Data are the mean difference between the low and the adequate vitamin D group, as defined in Column B. The mean differences, 95% confidence intervals (CIs), and corresponding p-values were estimated from generalized estimating equations, after adjustment for baseline measurements, follow-up time, and treatment (micronutrients *vs.* placebo) group.

[c] Multivariate analyses additionally adjusted for Age, Karnofsky Score, Baseline Hemoglobin, Viral Load, CD4 Count, and HIV Status; HIV status removed from the model where the results are stratified by HIV status. Viral Load also removed from the model in HIV-

uninfected individuals.





Supplemental Table 1	**Correlates of Low Vitamin D Status (25(OH)D < 75 nmol/L) in HIV-uninfected TB patients**					
		Univariate (p<0.20)		**Multivariate (p<0.05)**		
Variable		**RR (95% CI)**	**p-value**	**RR (95% CI)**	**p-value**
Season			**0.002**		**0.002**
	1: Dry (Jan-Feb)	Ref.		Ref.	
	2: Long Rains (Mar-Jun)	1.07 (0.74, 1.53)		1.09 (0.76, 1.56)	
	3: Dry (Jul-Oct)	1.50 (1.07, 2.09)		1.50 (1.08, 2.08)	
	4: Short Rains (Nov-Dec)	1.29 (0.84, 1.96)		1.26 (0.84, 1.90)	
Sex	Female	1.19 (0.99, 1.43)	0.07		
Cohabits with a partner		0.89 (0.75, 1.06)	0.19		
Money spent per person per day on food, per 1000 Tanzanian Shillings*		0.76 (0.59, 0.98)	**0.04**	0.76 (0.59, 0.98)	**0.03**
Money spent per person per day on food quartiles			0.14		
	0:<250	1.36 (1.03, 1.80)			
	1:250-499	1.11 (0.82, 1.52)			
	2:500-750	1.13 (0.84, 1.53)			
	3:>750	Ref.			
AFB Culture positive at baseline		1.17 (0.94, 1.46)	0.16		
Number of colonies in AFB culture			0.16		
	1	Ref.			
	2	1.01 (0.66, 1.56)			
	3	0.95 (0.63, 1.44)			
	4	1.05 (0.70, 1.58)			

	5		1.27 (1.01, 1.59)				
Received TB treatment in the past 5 years			1.41 (0.97, 2.04)	0.07			
Hemoglobin, g/dL			0.92 (0.87, 0.97)	**0.004**			
CD4 T-cells, 100 cells/µL			1.03 (0.99, 1.07)	0.12			
CD3 T-cells, 100 cells/µL			1.02 (1.00, 1.04)	0.12			
Depressed >2 weeks, ever			1.17 (0.97, 1.42)	0.10			
Dysentery			0.23 (0.04, 1.41)	0.11			
Outpatient visit			1.14 (0.95, 1.38)	0.16			
Skin rash			0.73 (0.46, 1.16)	0.18			
Height quartiles, cm				**0.01**			**0.01**
	<158.1		Ref.			Ref.	
	158.1-164.0		1.08 (0.87, 1.32)			1.12 (0.93, 1.36)	
	164.1-169.5		0.76 (0.59, 0.98)			0.82 (0.64, 1.04)	
	169.6+		0.77 (0.60, 0.99)			0.81 (0.64, 1.03)	
Weight, kg			0.99 (0.97, 1.00)	**0.03**			
WHO BMI groups, kg/m^2				0.14			
	<16		1.14 (0.88, 1.48)				
	16-16.99		0.98 (0.74, 1.29)				
	17-18.49		Ref.				
	18.5-19.99		0.76 (0.57, 1.02)				
	20-21.99		0.89 (0.68, 1.15)				
	22+		1.11 (0.83, 1.48)				
Mid-Upper Arm Circumference (MUAC) <22 cm			1.27 (1.07, 1.51)	**0.01**			
Triceps Skinfold Thickness,			1.02 (1.00, 1.03)	0.09			

cm							

p-values obtained using Binomial Regression; RR: Risk Ratio; 95% CI: 95% Confidence Interval; *1 US Dollar ≅ 1000 Tanzanian Shillings at the time of the study

Online Supporting Material

Supplemental Table 2	**Correlates of Low Vitamin D Status (serum 25(OH)D <75 nmol/L) in HIV-infected TB patients**					
		Univariate (p<0.20)		**Multivariate (p<0.05)**		
Variable		**RR (95% CI)**	**p-value**		**RR (95% CI)**	**p-value**
Age		0.99 (0.98, 1.00)	**0.01**			
Center			**0.03**			
	Mwananyamala	1.05 (0.86, 1.27)				
	Temeke	Ref.				
	Tandale	0.80 (0.63, 1.02)				
	Mbgala	0.67 (0.44, 1.01)				
	Amana	1.03 (0.82, 1.29)				
Cohabits with a partner		0.90 (0.77, 1.05)	0.19			
Assets at home			0.07			
	0:none	1.36 (1.03, 1.80)				
	1:one	0.88 (0.66, 1.16)				
	2:2-3	0.87 (0.67, 1.14)				
	3:4-5	Ref.				
Received TB treatment in the past 5 years		0.58 (0.31, 1.10)	0.10			
Hemoglobin, g/dL		0.95 (0.90, 1.00)	**0.04**		0.93 (0.89, 0.98)	**0.007**
Albumin, U/L		0.93 (0.86, 1.02)	0.13			
CD4 T-cells, 100 cells/µL		1.02 (0.99, 1.05)	0.12		1.04 (1.01, 1.07)	**0.02**
Depressed >2 weeks, ever		0.80 (0.61, 1.05)	0.11			
Hospitalization		1.30 (0.94, 1.80)	0.11			
Skin rash		1.32 (1.07, 1.64)	**0.01**			
Extrapulmonary TB		1.35 (0.93, 1.96)	0.11			

Height, cm			0.99 (0.98, 1.00)	0.08			
Weight, kg			0.99 (0.98, 1.00)	0.11			
WHO Body Mass Index (BMI) groups, kg/m^2				0.06			
	<16		0.81 (0.57, 1.16)				
	16-16.99		0.99 (0.76, 1.27)				
	17-18.49		Ref.				
	18.5-19.99		0.80 (0.63, 1.02)				
	20-21.99		0.75 (0.58, 0.96)				
	22+		1.05 (0.84, 1.30)				
Mid-Upper Arm Circumference (MUAC) quartiles, cm				**0.02**			
	<=21		Ref.				
	21.1-23.0		1.09 (0.90, 1.32)				
	23.1-24.9		0.81 (0.61, 1.08)				
	25+		0.82 (0.66, 1.03)				

p-values obtained using Binomial Regression; RR: Risk Ratio; 95% CI: 95% Confidence Interval

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2-3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	5
Objectives	3	State specific objectives, including any prespecified hypotheses	6
Methods			
Study design	4	Present key elements of study design early in the paper	6-8
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6-8
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	6-8
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6, 9
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	9-10
Bias	9	Describe any efforts to address potential sources of bias	10
Study size	10	Explain how the study size was arrived at	10
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	10
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	9-10
		(*b*) Describe any methods used to examine subgroups and interactions	9-10
		(*c*) Explain how missing data were addressed	9-10
		(*d*) If applicable, explain how loss to follow-up was addressed	9-10
		(*e*) Describe any sensitivity analyses	NA
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	10, Tables
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	10-11
		(b) Indicate number of participants with missing data for each variable of interest	10
		(c) Summarise follow-up time (eg, average and total amount)	11
Outcome data	15*	Report numbers of outcome events or summary measures over time	Tables
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Tables
		(*b*) Report category boundaries when continuous variables were categorized	Tables
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	10-13
Discussion			
Key results	18	Summarise key results with reference to study objectives	13
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	14-19
Generalisability	21	Discuss the generalisability (external validity) of the study results	18-19
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	20

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ open

Vitamin D Status and TB Treatment Outcomes in Adult Tanzanian Patients

Journal:	*BMJ Open*
Manuscript ID:	bmjopen-2013-003703.R1
Article Type:	Research
Date Submitted by the Author:	29-Sep-2013
Complete List of Authors:	Mehta, Saurabh; Cornell University , Mugusi, Ferdinand; Muhimbili University of Health and Allied Sciences, Bosch, Ronald; Harvard University, Aboud, Said; Muhimbili University of Health and Allied Sciences, Urassa, Willy; World Health Organization, Villamor, Eduardo; University of Michigan Ann Arbor, Fawzi, Wafaie; Harvard School of Public Health,
Primary Subject Heading:	Global health
Secondary Subject Heading:	Infectious diseases, HIV/AIDS, Nutrition and metabolism, Epidemiology, Respiratory medicine
Keywords:	Tuberculosis < INFECTIOUS DISEASES, HIV , Africa , Vitamin D

Vitamin D Status and TB Treatment Outcomes in Adult Tanzanian Patients

Saurabh Mehta[1], Ferdinand M. Mugusi[2], Ronald J. Bosch[3], Said Aboud[4], Willy Urassa[5], Eduardo Villamor[6], Wafaie W. Fawzi[7]

Corresponding Author: Saurabh Mehta, M.B.B.S., Sc.D.

Division of Nutritional Sciences, Cornell University

314 Savage Hall, Ithaca NY 14853

Phone: +1-607-255-2640; Fax: +1-607-255-1033; E-mail: smehta@cornell.edu

Word Count: 3743 words

Number of figures: 2

Number of tables: 5

Online Supplementary Information: 2 tables

Running Title: Vitamin D and Tuberculosis

Keywords: Vitamin D, Tuberculosis, HIV, Africa

[1] Division of Nutritional Sciences, Cornell University, Ithaca NY 14853
[2] Department of Internal Medicine, Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania
[3] Department of Biostatistics, Harvard School of Public Health, Boston MA 02115
[4] Department of Microbiology and Immunology, Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania
[5] Diagnostics and Laboratory Technology Team, World Health Organization, Geneva, Switzerland
[6] Department of Epidemiology, University of Michigan School of Public Health, Ann Arbor MI 48109
[7] Departments of Global Health and Population, Nutrition, and Epidemiology, Harvard School of Public Health, Boston MA 02115

Abstract

Objectives: Vitamin D is an immunomodulator and can alter response to tuberculosis treatment, though randomized trials have been inconclusive to date. We present the first comprehensive analysis of the associations between vitamin D status and TB treatment, T-cell counts, and nutritional outcomes by HIV status.

Design: Cohort study

Setting: Outpatient clinics in Tanzania

Participants: 25-hydroxyvitamin D levels were assessed in a cohort of 677 patients with TB (344 HIV-infected) initiating anti-TB treatment at enrollment in a multivitamin supplementation (excluding vitamin D) trial (Clinicaltrials.gov identifier: NCT00197704).

Primary and secondary outcome measures: Information on treatment outcomes such as failure and relapse, HIV disease progression, T-cell counts, and anthropometry was collected routinely, with a median follow-up of 52 and 30 months for HIV-uninfected and HIV-infected patients, respectively. Cox and binomial regression, and generalized estimating equations were used to assess the association of vitamin D status with these outcomes.

Results: Mean 25-hydroxyvitamin D concentrations at enrollment were 69.8 (±21.5) nmol/L [27.9 (±8.6) ng/mL]. Vitamin D insufficiency (<75 nmol/L) was associated with a 66% higher risk of relapse (95% CI: 4%, 164%; 133% higher risk in HIV-uninfected patients). Each unit higher vitamin D levels at baseline were associated with a decrease of 3 (p=0.004) CD8 and 3 (p=0.01) CD3 T-cells/µL during follow-up in HIV-infected patients. Vitamin D insufficiency was also associated with a greater decrease of BMI (-0.21 kg/m^2; 95% CI:-0.39, -0.02), during the first eight months of follow-up. No association was observed for vitamin D status with mortality or HIV disease progression.

Conclusions: Adequate vitamin D status is associated with a lower risk of relapse and with improved nutritional indicators such as BMI in TB patients, with or without HIV infection. Further research is needed to determine the optimal dose of vitamin D and effectiveness of daily vitamin D supplementation among patients with TB.

Article Focus: Recent laboratory data has suggested that optimal vitamin D status may be associated with a more effective immune response to TB infection, a faster rate of bacteriologic cure, and better long-term outcomes. However, clinical and epidemiological studies have found inconsistent results. In this paper, we present the first comprehensive analysis of the associations between vitamin D status and TB treatment, T-cell counts, and nutritional outcomes by HIV status.

Key Messages: We found that patients with adequate vitamin D status were less likely to experience a relapse during follow-up after completing TB treatment. They were also more likely to have a better nutritional status, as assessed by their body mass index, during follow-up, compared to patients with vitamin D insufficiency. The results provide justification for conducting both a dose response study to determine optimal dose of vitamin D and a randomized controlled trial of daily vitamin D supplementation among patients with TB.

Strengths and Limitations of this study: The major strengths of this study include a large number of participants, more than half of whom were HIV-infected, comprehensive assessment of clinical, immunological, socio-demographic, and nutritional parameters, and a long duration of follow-up. On the other hand, the major limitation is the possibility of reverse causation and residual confounding. We have attempted to minimize this through rigorous analyses and adjusting for several potential confounders, including hemoglobin concentrations, HIV status, viral load, CD4 T-cells, and Karnofsky score, in most analyses.

Introduction

Mycobacterium tuberculosis is one of the most pernicious infectious diseases and successful pathogens known to man. More than 95% of the estimated cases and deaths due to tuberculosis (TB) occur in low-income countries. The United Republic of Tanzania is one of the 22 high-burden countries that account for 80% of global TB cases. Tanzania has an incidence of 177 cases per 100,000 population per year and a prevalence of 183 cases per 100,000 population per year [1].

The spread of Human Immunodeficiency Virus (HIV) has fuelled the resurgence of the TB epidemic in Tanzania, as in other parts of sub-Saharan Africa [2]. HIV is the strongest factor in the development of active TB; it is estimated that only one out of ten immunocompetent persons infected with TB develops active TB in his/her lifetime; whereas, one out of ten HIV-infected persons infected with TB will develop active TB every year. An estimated 38% of TB patients in Tanzania are also infected with HIV [1].

Current treatment regimens, given under appropriate management conditions, are nearly 100% curative for patients with drug-susceptible organisms. However, in Tanzania, treatment fails in 12-17% of the cases. Additionally, TB patients in settings such as Tanzania grapple with multiple health-related and quality of life issues, which are not addressed adequately with treatment alone.

Recent data has suggested that optimal vitamin D status may be associated with a more effective immune response to TB infection, a faster rate of bacteriologic cure, and better

long-term outcomes. For example, a recent cross-sectional study found that vitamin D deficiency is highly prevalent in South Africa and is associated with susceptibility to active TB both in the presence and absence of HIV infection [3]. A few randomized trials have also been conducted; two of the recent ones failed to find an effect of vitamin D supplementation on treatment success [4 5]. However, the dose used and duration of supplementation may have precluded finding an effect. Further, most studies had small sample sizes and assessed only a limited number of covariates.

In this manuscript, we comprehensively examined the hypotheses that vitamin D status may be associated with response to treatment, risk of treatment failure, laboratory parameters such as T-cell counts, and anthropometric measurements in the context of a randomized trial of micronutrient supplementation (supplement did not contain vitamin D) in Tanzania to better inform future studies or trials.

Materials and Methods

Study Population: The study population and recruitment methods have been described in detail earlier [6]. Briefly, 887 adults with pulmonary tuberculosis (PTB) were enrolled in a randomized trial (Clinicaltrials.gov identifier: NCT00197704) to examine the effects of micronutrient supplementation on TB treatment failure, relapse, and mortality. The trial started in April 2000 in Dar es Salaam, Tanzania and continued until April 2005. The eligibility criteria for the study included positive sputum smears for acid-fast bacilli (AFB), age between 18 and 65 years, Karnofsky performance score of ≥ 40% [7], plan to stay in Dar es Salaam for 2 years, not being pregnant, and not having received anti-TB

treatment during the previous one year. Consenting subjects were randomly assigned in computer-generated permuted blocks of 20, stratified by HIV status, to receive a daily oral dose of 1 of 2 regimens: micronutrients (5000 IU of retinol, 20 mg of vitamin B1, 20mg of vitamin B2, 25mg of vitamin B_6, 100 mg of niacin, 50 µg of vitamin B_{12}, 500 mg of vitamin C, 200 mg of vitamin E, 0.8 mg of folic acid, and 100 µg of selenium) or placebo. These doses represent between 6 and 10 times the recommended dietary allowance (RDA) and were being tested at the time among HIV-infected adults from this setting [8]. We chose multiples of the RDA because previous observational studies suggested that HIV-infected individuals need higher dietary intakes of micronutrients to achieve normal serum concentrations [9]. All patients received a daily combination of rifampicin, isoniazid, pyrazinamide, and ethambutol under direct observation of a health worker during the first 2 months (intensive phase) followed by 6 months of self-administered daily isoniazid and ethambutol, as per the Tanzania National TB and Leprosy Programme guidelines. None of the HIV-infected patients received antiretroviral therapy, as these medications were not routinely available in Tanzania at the time this trial was conducted.

At the time of randomization, research nurses collected information on various socio-demographic characteristics including age, education levels, marital status, and socioeconomic status. Anthropometric measurements were also obtained using standardized procedures [10] at the randomization visit as well as during each monthly follow-up visit. Height was measured to the nearest 0.1 cm using SECA Bodymeter 206 stadiometers, weight to the nearest 100 g with SECA 700 balance beam scales, and left

mid-upper arm circumference (MUAC) at the midpoint between the acromion and olecranon to the nearest 0.1 cm using non-stretchable tailor's tapes.

Physician visits were scheduled every 3 months. During these visits, study physicians inquired about the health of the subject during the preceding period and performed a complete physical examination. The stage of HIV disease was assessed according to the World Health Organization system [11].

Ethics Approval: A written informed consent was obtained from all the study participants. The institutional review boards of the Muhimbili University of Health and Allied Sciences, the Tanzanian National AIDS Control Program, and the Harvard School of Public Health approved the study protocol.

Laboratory Methods: At the time of initiation of anti-TB treatment, HIV status was assessed among consenting patients using 2 sequential ELISAs (Wellcozyme, Murex Biotech; Enzygnost anti-HIV1/2 Plus, Dade Behring); discrepant results were resolved by Western Blot test (Bio-rad, Genetic Systems). Both pre-test and post-test counseling was provided. A blood sample also was obtained for measurement of hemoglobin and albumin concentrations using AcT Diff II hematology analyzer (Beckman Coulter, Miami) and Hitachi 911 analyzer (Roche Diagnostics), respectively. CD4, CD3, and CD8 T-cell counts were determined using FACScount or FACSCan systems (Becton Dickinson, CA, USA). Viral load was also determined using the Amplicor HIV-1 monitor v1.5 assay (Roche Molecular Systems, Branchburg, NJ, USA).

Assessment of Vitamin D Status: Serum 25-hydroxyvitamin D concentrations were measured using liquid chromatography-mass spectrometry at the Children's Hospital in Boston only at enrollment before the initiation of micronutrient supplementation. We defined vitamin D insufficiency status as serum 25(OH)D levels of less than 75 nmol/L and adequate otherwise. Vitamin D deficiency was defined as serum 25(OH)D levels of less than 50 nmol/L.

Statistical Analysis: We examined the association of vitamin D status with TB treatment outcomes as well as nutritional, immunological, and clinical end points in the entire cohort and separately by HIV status at baseline. TB-related end points included treatment failure, early relapse, and late relapse. Treatment failure by 1 month was defined as positive AFB cultures at 1 month from the initiation of treatment. Relapses were deemed to have occurred in patients with positive cultures, among those who had become culture negative after treatment initiation. Relapses/recurrences included both endogenous reactivation and exogenous reinfection, which could not be distinguished in this study. We calculated the relative risks (RRs) and 95% confidence intervals (CIs) for each of these outcomes by vitamin D status using binomial regression. We used Cox proportional hazards models to assess the association of vitamin D status with mortality in all patients and HIV disease progression from stage 3 to 4 in HIV-infected participants. We defined the end of follow-up as the date when HIV stage was last assessed.

We examined the association of vitamin D status with CD4, CD8, and CD3 T-cell counts, viral load (in HIV-infected participants), indicators of nutritional status (body mass index [BMI] and albumin concentrations), and hemoglobin concentrations using generalized estimating equations (GEEs). These models do not require that all patients have the same number of follow-up assessments or that the follow-up measurements be obtained at exactly the same time points. We assumed a standard normal distribution for repeated continuous end points (T cell subsets, $\log_{10}$ viral load, anthropometry, and albumin and hemoglobin concentrations) and estimated average differences during follow-up by vitamin D status. We used an exchangeable correlation structure to account for within-subject correlations and adjusted the models for the follow-up time when the measurements had been obtained and for the baseline values.

We analyzed the data for the entire period and for the first 8 months, coinciding with the expected end of TB treatment. Multivariate analyses adjusted for age, Karnofsky score, baseline hemoglobin concentrations, viral load, HIV status, CD4 T-cell counts, and micronutrient supplementation, unless otherwise specified in the results section or the tables. All analyses were performed using SAS software version 9.3 (SAS Institute Inc., Cary NC).

Results

Baseline 25-hydroxyvitamin D concentrations were available for 677 patients out of the original cohort of 887. Mean 25-hydroxyvitamin D concentration was 69.8 (±21.5) nmol/L [27.9 (±8.6) ng/mL] and its distribution is shown in Figure 1. The baseline

characteristics of these 677 patients by HIV status are presented in Table 1. 36% of the HIV-infected patients had CD4 T-cell counts below 200 cells/μL. The mean body mass index (BMI) was 19.1 ± 2.7 kg/m^2. The median follow-up time for HIV-uninfected patients was 52 months (inter-quartile range [IQR]: 47-57 months) and for HIV-infected patients was 30 months (IQR: 15-41 months).

The mean 25-hydroxyvitamin D concentrations were significantly different across season of blood draw in this cohort (p=0.004). Tanzania has four seasons: dry (January-February); long rains (March-June); dry (July-October); short rains (November-December). The boxplot of vitamin D's association with season of blood draw is presented in Figure 2. In subgroup analyses, this association was only observed among the HIV-uninfected patients and not the HIV-infected patients.

We examined the correlates of vitamin D insufficiency, defined as serum 25(OH)D concentrations below 75 nmol/L (75 nmol/L) in Supplemental Tables 1 (HIV-uninfected) and 2 (HIV-infected). All factors that had univariate associations with p<0.20 were included in a multivariate model; only the factors that had p<0.05 were retained in the final model. Among the HIV-uninfected subset, patients enrolled in the dry winter season between July and October were 50% more likely to have vitamin D insufficiency, compared to patients enrolled in the dry summer season between January and February (p for season=0.002). Similarly, the participants with the lowest height were more likely to have vitamin D insufficiency (p=0.01). Finally, greater expenditure on food per person per day was associated with a lower risk of having inadequate vitamin D status (Risk

Ratio [RR] per 1000 Tanzanian Shillings [approximately 1 US Dollar at the time of the study]: 0.76; 95% Confidence Interval [CI]: 0.59, 0.98). In the HIV-infected subset, patients with higher hemoglobin concentrations at baseline were less likely to have vitamin D insufficiency, with a 7% lower risk per 1 g/dL higher hemoglobin level (p=0.007). On the other hand, higher number of CD4 T-cells was associated with a higher risk of having inadequate vitamin D status (4% higher risk per 100 CD4 T-cells/µL; p=0.02).

There was no significant association of vitamin D status at TB treatment initiation with mortality or HIV disease progression in this cohort (Table 2 includes only HIV-infected participants as there were only 13 deaths in the HIV-uninfected subset). There was no association observed between vitamin D status and treatment failure one month after initiation of TB treatment (Table 3). However, patients with vitamin D insufficiency (<75 nmol/L) had a 66% higher risk of relapse after becoming culture-negative at one month after initiation of TB treatment (95% CI: 4%, 164%). This association was more pronounced in those who were not HIV-infected at enrollment in the study (RR: 2.33; 95% CI: 1.26, 4.29). In analysis with continuous vitamin D levels, each nmol/L increase was associated with a 1% lower risk of relapse during follow-up (p=0.04).

Vitamin D insufficiency was observed to have no association with CD4 T-cell counts during the entire follow-up in either the HIV-infected or the HIV-uninfected subsets (Table 4). However, vitamin D insufficiency was associated with greater CD4 T-cell

counts during the first eight months of follow-up in the HIV-infected patients (mean difference: 58; 95% CI: 13, 104).

In analysis among HIV-infected patients with continuous vitamin D levels, each nmol/L higher vitamin D concentration was associated with a decrease of 3 CD8 and 3 CD3 T-cells per μL. Vitamin D insufficiency was associated with an average of 85 higher CD8 T-cells/μL during follow-up (95% CI: 4, 165). Similar results were observed when we restricted the analyses to the first eight months of follow-up, the duration of TB treatment at the time of the study in Tanzania. No relationship was observed with mean viral loads during follow-up in the patients who were HIV-infected at the time of enrollment.

In analysis examining association of vitamin D status with nutritional parameters in the entire period of follow-up, no significant relationship was observed with BMI, albumin, or hemoglobin concentrations (Table 5). During the first eight months of follow-up, patients with Vitamin D Insufficiency experienced a decline in BMI (Mean: -0.21 kg/m^2; 95% CI: -0.39, -0.02), compared to patients with adequate vitamin D status. These results were more pronounced in HIV-uninfected patients (Mean: -0.34; 95% CI: -0.60, -0.09) and not significant in HIV-infected patients. HIV-infected patients with vitamin D insufficiency had increased albumin levels (Mean: 0.94; 95% CI: 0.55, 1.32) during the first eight months of follow-up compared to patients with adequate vitamin D status.

Discussion

In this study among 677 patients with tuberculosis in Tanzania, more than 61% of the participants had 25-hydroxyvitamin D concentrations below 75 nmol/L (75 nmol/L). 25-hydroxyvitamin D concentrations were associated with the season of blood draw, money spent on food per person per day, and height in HIV-uninfected participants and hemoglobin concentrations and CD4 T-cell counts among HIV-infected patients. Vitamin D insufficiency (<75 nmol/L) was not associated with mortality, HIV disease progression, or treatment failure during follow-up in the entire cohort. However, patients with vitamin D insufficiency had an increased risk of experiencing TB relapse during follow-up. Further, vitamin D insufficiency was associated with a decline in CD8 and CD3 T-cells in both the first eight months (the duration of TB treatment) and the entire period of follow-up. A similar relationship was observed with BMI in the first eight months of follow-up.

Our study was conducted in Dar es Salaam, the largest urban center in Tanzania, and just six degrees south of the Equator. The prevalence of vitamin D insufficiency (>61%) in this study is higher than the approximately 40% found in a previous study among TB patients in Mwanza, Tanzania [12] and in our studies among HIV-infected pregnant women (~85% of them had stage 1 HIV disease, unlike this study) in Dar es Salaam [13 14]. However, this prevalence is lower than what was observed in a cross-sectional study in South Africa, where 88% of HIV-uninfected and 97% of HIV-infected TB patients had vitamin D insufficiency. The mean vitamin D concentration in this study was 69.8 nmol/L, compared to 86.5 nmol/L in the study in Mwanza and 28.8-40 nmol/L in the South African study. One study from Thailand observed similar levels (69.0 nmol/L) in

TB patients [15]; Thailand is located at a similar distance from the Equator as Tanzania, though it is in the northern hemisphere. Similar to the study in South Africa, the vitamin D levels were lowest in our study in the dry winter season between July and October, though the differences were not as stark. For example, the mean vitamin D concentration in January to March in the South African study was 56.8 nmol/L and 30.8 nmol/L between July and September, whereas in our study, the concentrations were 74.8 nmol/L for January through February, and 66.3 nmol/L for July through October.

Vitamin D is synthesized in the skin through the action of ultraviolet light on 7-dehydrocholesterol. Fatty fish, such as salmon and sardines, are good sources of vitamin D in the diet but are not widely available everywhere and are usually expensive. Increasing urbanization and a tendency to spend most time indoors are major factors that contribute to the inability of the skin to synthesize adequate amounts of vitamin D [16-18]. Additionally, the TB disease itself and/or the HIV co-infection in the participants in this study are probably the primary reasons for restricted physical activity, lack of adequate exposure to sunlight, and consequent low concentrations of vitamin D.

Several other investigators have examined correlates of vitamin D status in TB patients. The study in Mwanza found that marital status, BMI, and serum transferrin receptor concentrations were correlated with vitamin D status. Though the first two were correlated with vitamin D status in our study in univariate analyses, neither remained significant in multivariate analyses. We didn't measure serum transferrin receptor in our study, though we did observe a correlation of vitamin D status with hemoglobin

concentrations among the HIV-infected subset. Another study in South Africa found that TB status (active disease *vs.* latent infection), month of sampling, and BMI were significantly correlated with vitamin D status in multivariate analyses [3]. All patients in our study had active disease, and we didn't observe a relationship with BMI in our analyses. The study in South Africa incorporated only those correlates associated with serum 25(OH)D concentration with $P < 0.05$ in univariate analysis in the multivariate model. This may have precluded selection of important covariates and confounders, if measured, and produced biased estimates and confidence intervals; increasing the nominal significance level to 20% or more, as used in this study [19] can eliminate most of this bias. Most other studies have been with smaller sample sizes and have examined a limited set of covariates, compared to the current study.

There was no association of vitamin D status with mortality or HIV disease progression in this cohort, unlike our previous studies among HIV-infected pregnant women [13 20] or HIV-infected adults [21 22] in Tanzania. The major difference is that in our earlier studies [13 20], a large majority (~85%) of the participants had stage 1 or asymptomatic HIV disease, whereas in this study, most of the individuals were already at stage 3 disease.

The association of vitamin D insufficiency with TB recurrence/relapse, primarily driven by the HIV-uninfected subset, is a novel finding in a longitudinal study and has important implications. Vitamin D deficiency has been linked to TB in several studies – a hypothesis perhaps initially generated by the observed seasonality of TB. *In vitro* and

animal studies indicate that 1,25-dihydroxyvitamin D_3, the most active form of vitamin D, may increase mycobacterial killing by macrophages but also limits host damage by decreasing the gamma-interferon production [23-28]. In perhaps the strongest evidence to date for a role of vitamin D in tuberculosis, a study by Liu *et al* [29] found that the antimycobacterial response in humans is dependent on adequate availability of vitamin D.

A few randomized trials of vitamin D supplementation in TB patients have been conducted in the past few years [4 5 30]. In a randomized trial that was conducted among 365 TB patients in Guinea-Bissau starting antituberculosis treatment, overall mortality was 15% (54 of 365) at 1 year of follow-up and similar in both arms [5]. Martineau and colleagues didn't find a difference in median time to sputum culture conversion with vitamin D supplementation of 2.5 mg vitamin D3 at enrollment, 14, 28, and 42 days after starting TB treatment in 126 adults with sputum smear-positive PTB [4]. A recent report by Coussens *et al* from a subset of the 126 adults included in the trial above stated that median time to sputum smear conversion in the intervention arm was significantly shorter than in the control arm (23 *vs.* 36 days; p=0.04) [30]. The lack of effect and concordance in most of these trials is probably due to the dose and dosing interval used. It is worth noting however, that large intermittent doses of vitamin D may result in supraphysiological concentrations in some cases, which may be more harmful than helpful in their effects on the immune system [31].

Vitamin D insufficiency also was associated with T-cell subset counts only among the HIV-infected patients in this cohort. We can only speculate as to the reasons for the significantly higher increase in CD4 T-cells observed in patients with vitamin D insufficiency at baseline. One potential explanation is that HIV-infected patients with vitamin D insufficiency may experience more uncontrolled immune reconstitution, leading to a greater increase in CD4 T-cell counts, on treatment of TB, compared to patients with adequate vitamin D status. This may also explain why this relationship is observed only in the first eight months of follow-up and not subsequently.

The results for CD8 and CD3 T-cells are consistent with our previous studies among HIV-infected women in Tanzania [13 14]. This could suggest a possible role of vitamin D in inflammation. Although, the conventional role of CD8 cells is as cytotoxic cells, they also are effector cells in inflammation [32]. The involvement of vitamin D in modulating CD8 cells is also indicated by the fact that CD8 cells express the highest concentration of vitamin D receptor of the immune cells [33]. Other studies also have found that vitamin D suppresses antigen-stimulated proinflammatory cytokine responses, which may help speed up resolution of inflammatory responses that can lead to increased risk of mortality among TB patients [30].

TB, once known as ‘consumption’, is associated with significant wasting and weight loss. The observation that better vitamin D status among HIV-uninfected patients is associated with a greater increase in BMI during follow-up is likely related to decreased risk of

relapse among these patients, as well as improvement in quality of life through mechanisms such as better metabolism that were not directly assessed in this study. The major strengths of this study include a large number of participants, more than half of whom were HIV-infected, comprehensive assessment of clinical, immunological, socio-demographic, and nutritional parameters, and a long duration of follow-up. On the other hand, the major limitation is the possibility of reverse causation and residual confounding. We have attempted to minimize this through rigorous analyses and adjusting for several potential confounders, including hemoglobin concentrations, HIV status, viral load, CD4 T-cells, and Karnofsky score, in most analyses. The study results are generalizable to most settings with a high TB burden and widely prevalent vitamin D insufficiency.

In summary, our study results indicate that adequate vitamin D status is associated with better clinical and nutritional parameters during follow-up in a cohort of TB patients in Tanzania. While randomized trials of vitamin D supplementation among TB patients are urgently warranted, it is also imperative to conduct dose-response studies to determine ideal dose and duration for the supplement.

Acknowledgments

We thank the patients, whose participation made this study possible. We are grateful to the study coordinator, Dorothy Mallya, and to the study physicians, research nurses, and laboratory and administrative staff at Muhimbili University of Health and Allied Sciences in Dar es Salaam, Tanzania, for their contributions to the project.

Financial support

National Institute of Allergy and Infectious Diseases, National Institutes of Health (grant U01AI045441 to support the study)

The authors report NO conflict of interest

Author Contributions

SM wrote the first draft of the manuscript and analyzed and interpreted the data; FMM, RJB, SA, WU, EV, and WWF were investigators of the parent trial and contributed to field activities and oversight; RJB also helped with the analysis and interpretation of the data; all authors participated in study design and contributed to the final manuscript. All authors have also read and approved the final manuscript.

Data sharing

No additional data available on vitamin D and tuberculosis.

References

1. WHO. Global Tuberculosis Control. Geneva: World Health Organization, 2011.

2. Chaisson RE, Martinson NA. Tuberculosis in Africa--combating an HIV-driven crisis. N Engl J Med 2008;**358**(11):1089-92 doi: 358/11/1089 [pii] 10.1056/NEJMp0800809[published Online First: Epub Date]|.

3. Martineau AR, Nhamoyebonde S, Oni T, et al. Reciprocal seasonal variation in vitamin D status and tuberculosis notifications in Cape Town, South Africa. Proc Natl Acad Sci U S A 2011;**108**(47):19013-7 doi: 10.1073/pnas.1111825108[published Online First: Epub Date]|.

4. Martineau AR, Timms PM, Bothamley GH, et al. High-dose vitamin D(3) during intensive-phase antimicrobial treatment of pulmonary tuberculosis: a double-blind randomised controlled trial. Lancet 2011;**377**(9761):242-50 doi: 10.1016/S0140-6736(10)61889-2[published Online First: Epub Date]|.

5. Wejse C, Gomes VF, Rabna P, et al. Vitamin D as supplementary treatment for tuberculosis: a double-blind, randomized, placebo-controlled trial. American Journal of Respiratory and Critical Care Medicine 2009;**179**(9):843-50 doi: 10.1164/rccm.200804-567OC[published Online First: Epub Date]|.

6. Villamor E, Mugusi F, Urassa W, et al. A Trial of the Effect of Micronutrient Supplementation on Treatment Outcome, T Cell Counts, Morbidity, and Mortality in Adults with Pulmonary Tuberculosis. J Infect Dis 2008;**197**(11):1499-505 doi: 10.1086/587846 10.1086/587846 [pii][published Online First: Epub Date]|.

7. Karnofsky DA, Abelmann WH, Craver LF, et al. The use of nitrogen mustards in the palliative treatment of cancer. Cancer 1948;**1**:634-56

8. Fawzi WW, Msamanga GI, Spiegelman D, et al. A randomized trial of multivitamin supplements and HIV disease progression and mortality. N Engl J Med 2004;**351**(1):23-32

9. Baum M, Cassetti L, Bonvehi P, et al. Inadequate dietary intake and altered nutrition status in early HIV-1 infection. Nutrition 1994;**10**(1):16-20

10. Lohman TG, Roche AF, Martorell R. *Anthropometric standardization reference manual*. Champaign, IL: Human Kinetics Books, 1988.

11. WHO. Interim proposal for a WHO staging system for HIV infection and disease. Wkly Epidemiol Rec 1990;**65**:221-24

12. Friis H, Range N, Pedersen ML, et al. Hypovitaminosis D is common among pulmonary tuberculosis patients in Tanzania but is not explained by the acute phase response. J Nutr 2008;**138**(12):2474-80 doi: 138/12/2474 [pii] 10.3945/jn.108.094979[published Online First: Epub Date]|.

13. Mehta S, Giovannucci E, Mugusi FM, et al. Vitamin D status of HIV-infected women and its association with HIV disease progression, anemia, and mortality. PLoS ONE 2010;**5**(1):e8770 doi: 10.1371/journal.pone.0008770[published Online First: Epub Date]|.

14. Mehta S, Spiegelman D, Aboud S, et al. Lipid-soluble vitamins A, D, and E in HIV-infected pregnant women in Tanzania. European journal of clinical nutrition 2010;**64**(8):808-17 doi: 10.1038/ejcn.2010.76[published Online First: Epub Date]|.

15. Davies PD, Church HA, Bovornkitti S, et al. Altered vitamin D homeostasis in tuberculosis. Intern Med (Thailand) 1988;**4**:45-47

16. Norman AW. Nutritional Aspects of Vitamin D. http://vitamind.ucr.edu/nutri.html. Accessed Oct 9, 2006. 1999. http://vitamind.ucr.edu/nutri.html (accessed Oct 9, 2006).

17. Utiger RD. The need for more vitamin D. N Engl J Med 1998;**338**(12):828-9

18. Holick MF. High prevalence of vitamin D inadequacy and implications for health. Mayo Clin Proc 2006;**81**(3):353-73

19. Greenland S. Modeling and variable selection in epidemiologic analysis. Am J Public Health 1989;**79**(3):340-9

20. Mehta S, Mugusi FM, Spiegelman D, et al. Vitamin D status and its association with morbidity including wasting and opportunistic illnesses in HIV-infected women in Tanzania. AIDS Patient Care STDS 2011;**25**(10):579-85 doi: 10.1089/apc.2011.0182[published Online First: Epub Date]|.

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. The Journal of infectious diseases 2013;**207**(3):378-85 doi: 10.1093/infdis/jis693[published Online First: Epub Date]|.

22. Sudfeld CR, Wang M, Aboud S, et al. Vitamin D and HIV progression among Tanzanian adults initiating antiretroviral therapy. PloS one 2012;**7**(6):e40036 doi: 10.1371/journal.pone.0040036[published Online First: Epub Date]|.

23. Thoma-Uszynski S, Stenger S, Takeuchi O, et al. Induction of direct antimicrobial activity through mammalian toll-like receptors. Science 2001;**291**(5508):1544-7

24. Waters WR, Palmer MV, Nonnecke BJ, et al. Mycobacterium bovis infection of vitamin D-deficient NOS2-/- mice. Microb Pathog 2004;**36**(1):11-7

25. Waters WR, Nonnecke BJ, Foote MR, et al. Mycobacterium bovis bacille Calmette-Guerin vaccination of cattle: activation of bovine CD4+ and gamma delta TCR+ cells and modulation by 1,25-dihydroxyvitamin D3. Tuberculosis (Edinb) 2003;**83**(5):287-97

26. Waters WR, Nonnecke BJ, Rahner TE, et al. Modulation of Mycobacterium bovis-specific responses of bovine peripheral blood mononuclear cells by 1,25-dihydroxyvitamin D(3). Clin Diagn Lab Immunol 2001;**8**(6):1204-12

27. Kawakami K, Teruya K, Tohyama M, et al. [A therapeutic trial of experimental tuberculosis with gamma-interferon in an immunocompromised mouse model]. Kekkaku 1994;**69**(10):607-13

28. McMurray DN, Bartow RA, Mintzer CL, et al. Micronutrient status and immune function in tuberculosis. Ann N Y Acad Sci 1990;**587**:59-69

29. Liu PT, Stenger S, Li H, et al. Toll-Like Receptor Triggering of a Vitamin D-Mediated Human Antimicrobial Response. Science 2006:1123933 doi: 10.1126/science.1123933[published Online First: Epub Date]|.

30. Coussens AK, Wilkinson RJ, Hanifa Y, et al. Vitamin D accelerates resolution of inflammatory responses during tuberculosis treatment. Proc Natl Acad Sci 2012:Epub

31. Martineau AR. Bolus-dose vitamin D and prevention of childhood pneumonia. Lancet 2012;**379**(9824):1373-5 doi: 10.1016/S0140-6736(12)60405-X[published Online First: Epub Date]|.

32. Babbe H, Roers A, Waisman A, et al. Clonal expansions of CD8(+) T cells dominate the T cell infiltrate in active multiple sclerosis lesions as shown by micromanipulation and single cell polymerase chain reaction. J Exp Med 2000;**192**(3):393-404

33. Veldman CM, Cantorna MT, DeLuca HF. Expression of 1,25-dihydroxyvitamin D(3) receptor in the immune system. Arch Biochem Biophys 2000;**374**(2):334-8 doi: 10.1006/abbi.1999.1605 S0003-9861(99)91605-3 [pii][published Online First: Epub Date]|.

Figure Legends

1. Distribution of 25-hydroxyvitamin D concentrations at baseline (nmol/L)

2. Distribution of 25-hydroxyvitamin D concentrations by season of blood draw;

   Season 1: Dry (January-February); Season 2: Long Rains (March-June); Season 3: Dry (July-October); Season 4: Short Rains (November-December)

Tables

Table 1	**Baseline Characteristics of the Study Population (n=677)**		
		HIV-infected (n=344)	**HIV-uninfected (n=333)**
Variable		**Mean (Standard Deviation)**	**Mean (Standard Deviation)**
Age, years		34.4 ± 8.6	30.2 ± 9.2
Money spent on food per person per day, Tanzanian Shillings*		587.3 ± 445.9	580.1 ± 684.2
Hemoglobin, g/dL		9.9 ± 1.8	11.1 ± 1.7
Albumin, g/dL		2.8 ± 1.0	3.2 ± 1.1
CD3 T-cell count, cells/μL		1228.0 ± 608.5	1195.9 ± 404.8
CD4 T-cell counts, cells/μL		327.2 ± 246.2	709.2 ± 250.8
CD8 T-cell counts, cells/μL		826.9 ± 447.5	427.5 ± 188.2
Log(10) Viral Load, copies/mL		4.6 ± 1.0	N/A
Body Mass Index, kg/m^2		19.4 ± 2.8	18.8 ± 2.5
Mid-Upper Arm Circumference, cm		23.4 ± 2.7	23.1 ± 2.7
Follow-up time, days		916.8 ± 507.4	1532.9 ± 331.4
		n (%)	***n (%)***
Vitamin D insufficiency (serum 25-hydroxyvitamin D <75 nmol/L)		218 (63.4%)	200 (60.1%)

Vitamin D deficiency (serum 25-hydroxyvitamin D <50 nmol/L)		55 (16.0%)	51 (15.3%)
Sex			
	Male	203 (59.0%)	257 (77.2%)
	Female	141 (41.0%)	76 (22.8%)
Center			
	Mwananyamala	79 (23.0%)	88 (26.4%)
	Temeke	102 (29.7%)	83 (24.9%)
	Tandale	83 (24.1%)	91 (27.3%)
	Mbgala	31 (9.0%)	70 (21.0%)
	Amana	49 (14.2%)	1 (.3%)
Karnofsky Score <70%		45 (13.1%)	29 (8.7%)
Education Group			
	None	29 (8.4%)	36 (10.8%)
	Low <5years	35 (10.2%)	31 (9.3%)
	Primary 5-8 years	238 (69.2%)	233 (70.0%)
	Secondary/University	42 (12.2%)	33 (9.9%)

Cohabits with a Partner		200 (58.1%)	168 (50.5%)
Assets at home			
	None	92 (26.9%)	108 (32.4%)
	One	89 (26.0%)	85 (25.5%)
	2-3	122 (35.7%)	114 (34.2%)
	4-5	39 (11.4%)	26 (7.8%)
WHO HIV Disease Stage			
	3	240 (90.9%)	N/A
	4	24 (9.1%)	
CD4 T-cell categories, cells/μL			
	0-199	97 (35.9%)	0 (.0%)
	200-499	116 (43.0%)	69 (22.9%)
	500+	57 (21.1%)	232 (77.1%)
WHO BMI Group, kg/m^2			
	<16	26 (7.7%)	33 (9.9%)
	16-16.99	37 (10.9%)	45 (13.6%)
	17-18.49	73 (21.5%)	88 (26.5%)
	18.5-19.99	79 (23.3%)	70 (21.1%)
	20-21.99	77 (22.7%)	69 (20.8%)

	22+	47 (13.9%)	27 (8.1%)

*1 US Dollar ≅ 1000 Tanzanian Shillings at the time of the study

Table 2	**Vitamin D Status and Mortality and HIV Disease Progression in HIV-infected TB Patients**					
		Univariate		**Multivariate**		
Outcome	***n/N (%)***	***RR (95% CI)***	***p-value***	***RR (95% CI)***	***p-value***
Mortality					
Vitamin D insufficiency (<75 nmol/L)	61/218 (28.0%)	0.73 (0.50, 1.08)	0.12	0.70 (0.47, 1.04)	0.08
Adequate Vitamin D	43/126 (34.1%)				
Vitamin D deficient (<50 nmol/L)	20/55 (36.4%)	1.34 (0.82, 2.18)	0.25	0.91 (0.55 1.50)	0.71
Not deficient	84/289 (29.1%)				
Continuous Vitamin D (nmol/L)		1.00 (0.99, 1.01)	0.49	1.01 (1.00, 1.02)	0.15
HIV Disease Progression					

	Vitamin D insufficiency (<75 nmol/L)	46/150 (30.7%)	1.10 (0.67, 1.82)	0.71		1.08 (0.64, 1.82)	0.78
	Adequate Vitamin D	23/90 (25.6%)	Reference			Reference	
	Vitamin D deficient (<50 nmol/L)	14/34 (41.2%)	1.91 (1.05, 3.44)	**0.03**		1.48 (0.78, 2.82)	0.23
	Not deficient	55/206 (26.7%)	Reference			Reference	
	Continuous Vitamin D (nmol/L)		0.99 (0.98, 1.01)	0.30		1. 00 (0.99, 1.01)	0.57
p-values obtained using Cox Proportional Hazards Regression; RR: Risk Ratio; 95% CI: 95% Confidence Interval							
Multivariate analyses adjusted for Age, Karnofsky Score, Baseline Hemoglobin, Viral Load, HIV Status, CD4 Counts, and Micronutrient Supplementation							

Table 3	Vitamin D Status and Treatment Outcomes in TB Patients					
		Univariate		Multivariate		
Outcome	*n/N (%)*	*RR (95% CI)*	*p-value*	*RR (95% CI)*	*p-value*	
Treatment Failure by 1 month post-treatment initiation						
Vitamin D insufficiency (<75 nmol/L)	58/298 (19.5%)	1.06 (0.72, 1.55)	0.77	1.02 (0.70, 1.49)	0.93	
Adequate Vitamin D	34/185 (18.4%)					
Vitamin D deficient (<50 nmol/L)	15/75 (20.0%)	1.06 (0.65, 1.74)	0.82	1.13 (0.69, 1.86)	0.63	
Not deficient	77/408 (18.9%)					
Continuous Vitamin D (nmol/L)		1.00 (0.99, 1.01)	0.49	1. 00 (0.99, 1.01)	0.50	
Any Relapse (relapse after 1 month post-treatment initiation if culture negative at 1 month)						
Vitamin D insufficiency	51/227 (22.5%)	1.56 (0.98, 2.48)	0.06	1.66 (1.04, 2.64)	**0.03**	

	(<75 nmol/L)							
	Adequate Vitamin D	21/146 (14.4%)						
	Vitamin D deficient (<50 nmol/L)	13/56 (23.2%)		1.25 (0.73, 2.12)	0.41		1.40 (0.82, 2.39)	0.21
	Not deficient	59/317 (18.6%)						
	Continuous Vitamin D (nmol/L)			0.99 (0.98, 1.00)	0.06		0.99 (0.98, 1.00)	**0.04**

p-values obtained using Binomial Regression; RR: Risk Ratio; 95% CI: 95% Confidence Interval

Multivariate analyses adjusted for Age, Karnofsky Score, Baseline Hemoglobin, Viral Load, HIV Status, CD4 Counts, and Micronutrient Supplementation

Table 4 Vitamin D Status and T-cell Counts (cells/µL) in TB Patients

		CD4 T-cells				CD8 T-cells				CD3 T-cells			
Outcome		*Adequate vitamin D, mean (SD)[a]*	*Vitamin D insufficiency, mean difference (95% CI)[b]*	*Vitamin D insufficiency, adjusted mean difference (95% CI)[c]*	*p-value*	*Adequate vitamin D, mean (SD)[a]*	*Vitamin D insufficiency, mean difference (95% CI)[b]*	*Vitamin D insufficiency, adjusted mean difference (95% CI)[c]*	*p-value*	*Adequate vitamin D, mean (SD)[a]*	*Vitamin D insufficiency, mean difference (95% CI)[b]*	*Vitamin D insufficiency, adjusted mean difference (95% CI)[c]*	*p-value*

Entire follow-up: HIV-infected patients															
	Vitamin D insufficiency (<75 nmol/L)		300 (234)	17 (-23, 56)	21 (-18, 59)	0.29		902 (457)	88 (7, 169)	85 (4, 165)	**0.04**		1298 (635)	101 (-4, 206)	103 (-5, 212)	0.06
	Vitamin D deficient (<50 nmol/L)		333 (225)	21 (-34, 76)	30 (-26, 86)	0.29		957 (424)	105 (-9, 219)	114 (-6, 234)	0.06		1392 (595)	104 (-47, 255)	125 (-28, 279)	0.11
	Continuous Vitamin D (per nmol/L)			0 (-1, 1)	-1 (-1, 0)	0.26			-3 (-5, -1)	-3 (-5, -1)	**0.0004**			-3 (-5, -1)	-3 (-6, -1)	**0.01**
Entire follow-up: HIV-uninfected patients																
	Vitamin D		771	-2 (-	3 (-45,	0.9		508	-25 (-	-22 (-	0.2		1351	-37 (-	-28 (-	0.4

	insufficiency (<75 nmol/L)		(235)	49, 45)	51)	1		(209)	63, 14)	60, 17)	7		(400)	109, 35)	99, 44)	5
	Vitamin D deficient (<50 nmol/L)		781 (241)	-34 (-99, 30)	-34 (-101, 32)	0.31		500 (195)	-1 (-64, 62)	3 (-61, 67)	0.93		1354 (397)	-33 (-136, 71)	-28 (-134, 79)	0.61
	Continuous Vitamin D (per nmol/L)			0 (-1, 1)	0 (-1, 1)	0.97			0 (-1, 1)	0 (-1, 1)	0.83			0 (-1, 2)	0 (-2, 2)	0.90
First 8 months of follow-up: HIV-infected patients																
	Vitamin D insufficiency (<75 nmol/L)		316 (237)	54 (8, 100)	58 (13, 104)	**0.01**		868 (470)	132 (29, 235)	119 (15, 223)	**0.02**		1279 (670)	190 (42, 337)	179 (28, 331)	**0.02**

	Vitamin D deficient (<50 nmol/L)		372 (264)	36 (-25, 97)	41 (-20, 101)	0.19		963 (471)	63 (-77, 203)	75 (-72, 221)	0.32		1443 (689)	67 (-125, 259)	101 (-93, 295)	0.31
	Continuous Vitamin D (per nmol/L)			-1 (-2, 0)	-1 (-2, 0)	**0.01**			-4 (-6, -1)	-4 (-6, -1)	**0.002**			-4 (-7, -1)	-5 (-8, -2)	**0.003**
First 8 months of follow-up: HIV-uninfected patients															
	Vitamin D insufficiency (<75 nmol/L)		724 (243)	1 (-52, 53)	6 (-47, 59)	0.82		461 (232)	-22 (-63, 20)	-17 (-57, 22)	0.39		1248 (446)	-38 (-121, 46)	-27 (-106, 52)	0.50
	Vitamin D deficient		731	-7 (-	-7 (-	0.8		454	4 (-73,	5 (-71,	0.9		1247	14 (-	17 (-	0.8

	(<50 nmol/L)		(237)	95, 80)	96, 81)	7		(209)	81)	81)	0		(410)	124, 153)	123, 156)	2
	Continuous Vitamin D (per nmol/L)			0 (-2,1)	0 (-2,1)	0.54			0 (-1, 1)	0 (-1, 1)	0.73			0 (-2, 1)	0 (-2, 1)	0.57

[a] Data are the means (SD) of the average measurement during follow-up for each participant

[b] Data are the mean difference between the low and the adequate vitamin D group, as defined in Column B. The mean differences, 95% confidence intervals (CIs), and corresponding p-values were estimated from generalized estimating equations, after adjustment for baseline measurements, follow-up time, and treatment (micronutrients *vs.* placebo) group.

[c] Multivariate analyses additionally adjusted for Age, Karnofsky Score, and Baseline Hemoglobin

Table 5	Vitamin D Status and Nutritional Parameters in TB Patients													
	Body Mass Index (kg/m^2)					Albumin concentration (g/dL)					Hemoglobin concentration (g/dL)			
Outcome	*Adequate vitamin D, mean (SD)*[a]	*Vitamin D insufficiency, mean difference (95% CI)*[b]	*Vitamin D insufficiency, adjusted mean difference (95% CI)*[c]	*p-value*		*Adequate vitamin D, mean (SD)*[a]	*Vitamin D insufficiency, mean difference (95% CI)*[b]	*Vitamin D insufficiency, adjusted mean difference (95% CI)*[c]	*p-value*		*Adequate vitamin D, mean (SD)*[a]	*Vitamin D insufficiency, mean difference (95% CI)*[b]	*Vitamin D insufficiency, adjusted mean difference (95% CI)*[c]	*p-value*
Entire														

follow-up: All patients														
Vitamin D insufficiency (<75 nmol/L)	21.20 (2.80)	-0.06 (-0.30, 0.17)	-0.08 (-0.30, 0.14)	0.46		3.42 (0.74)	-0.05 (-0.14, 0.04)	0.00 (-0.08, 0.08)	0.97		12.65 (1.80)	-0.16 (-0.40, 0.08)	-0.18 (-0.41, 0.05)	0.12
Vitamin D deficient (<50 nmol/L)	21.23 (3.00)	-0.16 (-0.46, 0.14)	-0.14 (-0.44, 0.15)	0.34		3.42 (0.72)	-0.05 (-0.17, 0.07)	0.02 (-0.08, 0.13)	0.65		12.42 (1.87)	0.15 (-0.16, 0.45)	0.17 (-0.11, 0.45)	0.24

Continuous Vitamin D (per nmol/L)	0.00 (0.00, 0.01)	0.00 (0.00, 0.01)	0.30			0.002 (0.00, 0.004)	0.000 (-0.002, 0.002)	0.90			0.00 (-0.01, 0.01)	0.00 (0.00, 0.01)	0.85
First 8 months of follow-up: All patients													
Vitamin D insufficiency (<75 nmol/L)	20.96 (2.73)	-0.20 (-0.40, -0.01)	-0.21 (-0.39, -0.02)	**0.03**	3.42 (1.09)	-0.01 (-0.18, 0.16)	0.04 (-0.13, 0.21)	0.65	12.12 (1.85)	-0.01 (-0.28, 0.26)	-0.04 (-0.31, 0.23)	0.78	

Vitamin D deficient (<50 nmol/L)	20.85 (2.84)	0.00 (-0.25, 0.25)	0.04 (-0.21, 0.29)	0.78	3.41 (1.08)	-0.11 (-0.32, 0.10)	-0.05 (-0.27, 0.17)	0.64	11.92 (1.99)	0.16 (-0.18, 0.50)	0.21 (-0.10, 0.53)	0.19
Continuous Vitamin D (per nmol/L)		0.00 (0.00, 0.01)	0.00 (0.00, 0.01)	0.38		0.000 (-0.003, 0.005)	0.000 (-0.004, 0.004)	0.87		0.00 (-0.01, 0.00)	0.00 (-0.01, 0.00)	0.57

[a] Data are the means (SD) of the average measurement during follow-up for each participant

[b] Data are the mean difference between the low and the adequate vitamin D group, as defined in Column B. The mean differences, 95% confidence intervals (CIs), and corresponding p-values were estimated from generalized estimating equations, after adjustment for baseline

measurements, follow-up time, and treatment (micronutrients *vs.* placebo) group.
[c] Multivariate analyses additionally adjusted for Age, Karnofsky Score, Baseline Hemoglobin, Viral Load, CD4 Count, and HIV Status; HIV status removed from the model where the results are stratified by HIV status. Viral Load also removed from the model in HIV-uninfected individuals.

Vitamin D Status and TB Treatment Outcomes in Adult Tanzanian Patients

Saurabh Mehta[1], Ferdinand M. Mugusi[2], Ronald J. Bosch[3], Said Aboud[4], Willy Urassa[5], Eduardo Villamor[6], Wafaie W. Fawzi[7]

Corresponding Author: Saurabh Mehta, M.B.B.S., Sc.D.

Division of Nutritional Sciences, Cornell University

314 Savage Hall, Ithaca NY 14853

Phone: +1-607-255-2640; Fax: +1-607-255-1033; E-mail: smehta@cornell.edu

Word Count: 3743 words

Number of figures: 2

Number of tables: 5

Online Supplementary Information: 2 tables

Running Title: Vitamin D and Tuberculosis

Keywords: Vitamin D, Tuberculosis, HIV, Africa

[1] Division of Nutritional Sciences, Cornell University, Ithaca NY 14853
[2] Department of Internal Medicine, Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania
[3] Department of Biostatistics, Harvard School of Public Health, Boston MA 02115
[4] Department of Microbiology and Immunology, Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania
[5] Diagnostics and Laboratory Technology Team, World Health Organization, Geneva, Switzerland
[6] Department of Epidemiology, University of Michigan School of Public Health, Ann Arbor MI 48109
[7] Departments of Global Health and Population, Nutrition, and Epidemiology, Harvard School of Public Health, Boston MA 02115

Abstract

Objectives: Vitamin D is an immunomodulator and can alter response to tuberculosis treatment, though randomized trials have been inconclusive to date. We present the first comprehensive analysis of the associations between vitamin D status and TB treatment, T-cell counts, and nutritional outcomes by HIV status.

Design: Cohort study

Setting: Outpatient clinics in Tanzania

Participants: 25-hydroxyvitamin D levels were assessed in a cohort of 677 patients with TB (344 HIV-infected) initiating anti-TB treatment at enrollment in a multivitamin supplementation (excluding vitamin D) trial (Clinicaltrials.gov identifier: NCT00197704).

Primary and secondary outcome measures: Information on treatment outcomes such as failure and relapse, HIV disease progression, T-cell counts, and anthropometry was collected routinely, with a median follow-up of 52 and 30 months for HIV-uninfected and HIV-infected patients, respectively. Cox and binomial regression, and generalized estimating equations were used to assess the association of vitamin D status with these outcomes.

Results: Mean 25-hydroxyvitamin D concentrations at enrollment were 69.8 (±21.5) nmol/L [27.9 (±8.6) ng/mL]. Vitamin D insufficiency (<75 nmol/L) was associated with a 66% higher risk of relapse (95% CI: 4%, 164%; 133% higher risk in HIV-uninfected patients). Each unit higher vitamin D levels at baseline were associated with a decrease of 3 (p=0.004) CD8 and 3 (p=0.01) CD3 T-cells/µL during follow-up in HIV-infected patients. Vitamin D insufficiency was also associated with a greater decrease of BMI (-0.21 kg/m^2; 95% CI:-0.39, -0.02), during the first eight months of follow-up. No association was observed for vitamin D status with mortality or HIV disease progression.

Conclusions: Adequate vitamin D status is associated with a lower risk of relapse and with improved nutritional indicators such as BMI in TB patients, with or without HIV infection. Further research is needed to determine the optimal dose of vitamin D and effectiveness of daily vitamin D supplementation among patients with TB.

Article Focus: Recent laboratory data has suggested that optimal vitamin D status may be associated with a more effective immune response to TB infection, a faster rate of bacteriologic cure, and better long-term outcomes. However, clinical and epidemiological studies have found inconsistent results. In this paper, we present the first comprehensive analysis of the associations between vitamin D status and TB treatment, T-cell counts, and nutritional outcomes by HIV status.

Key Messages: We found that patients with adequate vitamin D status were less likely to experience a relapse during follow-up after completing TB treatment. They were also more likely to have a better nutritional status, as assessed by their body mass index, during follow-up, compared to patients with vitamin D insufficiency. The results provide justification for conducting both a dose response study to determine optimal dose of vitamin D and a randomized controlled trial of daily vitamin D supplementation among patients with TB.

Strengths and Limitations of this study: The major strengths of this study include a large number of participants, more than half of whom were HIV-infected, comprehensive assessment of clinical, immunological, socio-demographic, and nutritional parameters, and a long duration of follow-up. On the other hand, the major limitation is the possibility of reverse causation and residual confounding. We have attempted to minimize this through rigorous analyses and adjusting for several potential confounders, including hemoglobin concentrations, HIV status, viral load, CD4 T-cells, and Karnofsky score, in most analyses.

Introduction

Mycobacterium tuberculosis is one of the most pernicious infectious diseases and successful pathogens known to man. More than 95% of the estimated cases and deaths due to tuberculosis (TB) occur in low-income countries. The United Republic of Tanzania is one of the 22 high-burden countries that account for 80% of global TB cases. Tanzania has an incidence of 177 cases per 100,000 population per year and a prevalence of 183 cases per 100,000 population per year [1].

The spread of Human Immunodeficiency Virus (HIV) has fuelled the resurgence of the TB epidemic in Tanzania, as in other parts of sub-Saharan Africa [2]. HIV is the strongest factor in the development of active TB; it is estimated that only one out of ten immunocompetent persons infected with TB develops active TB in his/her lifetime; whereas, one out of ten HIV-infected persons infected with TB will develop active TB every year. An estimated 38% of TB patients in Tanzania are also infected with HIV [1].

Current treatment regimens, given under appropriate management conditions, are nearly 100% curative for patients with drug-susceptible organisms. However, in Tanzania, treatment fails in 12-17% of the cases. Additionally, TB patients in settings such as Tanzania grapple with multiple health-related and quality of life issues, which are not addressed adequately with treatment alone.

Recent data has suggested that optimal vitamin D status may be associated with a more effective immune response to TB infection, a faster rate of bacteriologic cure, and better

long-term outcomes. For example, a recent cross-sectional study found that vitamin D deficiency is highly prevalent in South Africa and is associated with susceptibility to active TB both in the presence and absence of HIV infection [3]. A few randomized trials have also been conducted; two of the recent ones failed to find an effect of vitamin D supplementation on treatment success [4 5]. However, the dose used and duration of supplementation may have precluded finding an effect. Further, most studies had small sample sizes and assessed only a limited number of covariates.

In this manuscript, we comprehensively examined the hypotheses that vitamin D status may be associated with response to treatment, risk of treatment failure, laboratory parameters such as T-cell counts, and anthropometric measurements in the context of a randomized trial of micronutrient supplementation (supplement did not contain vitamin D) in Tanzania to better inform future studies or trials.

Materials and Methods

Study Population: The study population and recruitment methods have been described in detail earlier [6]. Briefly, 887 adults with pulmonary tuberculosis (PTB) were enrolled in a randomized trial (Clinicaltrials.gov identifier: NCT00197704) to examine the effects of micronutrient supplementation on TB treatment failure, relapse, and mortality. The trial started in April 2000 in Dar es Salaam, Tanzania and continued until April 2005. The eligibility criteria for the study included positive sputum smears for acid-fast bacilli (AFB), age between 18 and 65 years, Karnofsky performance score of ≥ 40% [7], plan to stay in Dar es Salaam for 2 years, not being pregnant, and not having received anti-TB

treatment during the previous one year. Consenting subjects were randomly assigned in computer-generated permuted blocks of 20, stratified by HIV status, to receive a daily oral dose of 1 of 2 regimens: micronutrients (5000 IU of retinol, 20 mg of vitamin B1, 20mg of vitamin B2, 25mg of vitamin B_6, 100 mg of niacin, 50 µg of vitamin B_{12}, 500 mg of vitamin C, 200 mg of vitamin E, 0.8 mg of folic acid, and 100 µg of selenium) or placebo. These doses represent between 6 and 10 times the recommended dietary allowance (RDA) and were being tested at the time among HIV-infected adults from this setting [8]. We chose multiples of the RDA because previous observational studies suggested that HIV-infected individuals need higher dietary intakes of micronutrients to achieve normal serum concentrations [9]. All patients received a daily combination of rifampicin, isoniazid, pyrazinamide, and ethambutol under direct observation of a health worker during the first 2 months (intensive phase) followed by 6 months of self-administered daily isoniazid and ethambutol, as per the Tanzania National TB and Leprosy Programme guidelines. None of the HIV-infected patients received antiretroviral therapy, as these medications were not routinely available in Tanzania at the time this trial was conducted.

At the time of randomization, research nurses collected information on various socio-demographic characteristics including age, education levels, marital status, and socioeconomic status. Anthropometric measurements were also obtained using standardized procedures [10] at the randomization visit as well as during each monthly follow-up visit. Height was measured to the nearest 0.1 cm using SECA Bodymeter 206 stadiometers, weight to the nearest 100 g with SECA 700 balance beam scales, and left

mid-upper arm circumference (MUAC) at the midpoint between the acromion and olecranon to the nearest 0.1 cm using non-stretchable tailor's tapes.

Physician visits were scheduled every 3 months. During these visits, study physicians inquired about the health of the subject during the preceding period and performed a complete physical examination. The stage of HIV disease was assessed according to the World Health Organization system [11].

Ethics Approval: A written informed consent was obtained from all the study participants. The institutional review boards of the Muhimbili University of Health and Allied Sciences, the Tanzanian National AIDS Control Program, and the Harvard School of Public Health approved the study protocol.

Laboratory Methods: At the time of initiation of anti-TB treatment, HIV status was assessed among consenting patients using 2 sequential ELISAs (Wellcozyme, Murex Biotech; Enzygnost anti-HIV1/2 Plus, Dade Behring); discrepant results were resolved by Western Blot test (Bio-rad, Genetic Systems). Both pre-test and post-test counseling was provided. A blood sample also was obtained for measurement of hemoglobin and albumin concentrations using AcT Diff II hematology analyzer (Beckman Coulter, Miami) and Hitachi 911 analyzer (Roche Diagnostics), respectively. CD4, CD3, and CD8 T-cell counts were determined using FACScount or FACSCan systems (Becton Dickinson, CA, USA). Viral load was also determined using the Amplicor HIV-1 monitor v1.5 assay (Roche Molecular Systems, Branchburg, NJ, USA).

Assessment of Vitamin D Status: Serum 25-hydroxyvitamin D concentrations were measured using liquid chromatography-mass spectrometry at the Children's Hospital in Boston only at enrollment before the initiation of micronutrient supplementation. We defined vitamin D insufficiency status as serum 25(OH)D levels of less than 75 nmol/L and adequate otherwise. Vitamin D deficiency was defined as serum 25(OH)D levels of less than 50 nmol/L.

Statistical Analysis: We examined the association of vitamin D status with TB treatment outcomes as well as nutritional, immunological, and clinical end points in the entire cohort and separately by HIV status at baseline. TB-related end points included treatment failure, early relapse, and late relapse. Treatment failure by 1 month was defined as positive AFB cultures at 1 month from the initiation of treatment. Relapses were deemed to have occurred in patients with positive cultures, among those who had become culture negative after treatment initiation. Relapses/recurrences included both endogenous reactivation and exogenous reinfection, which could not be distinguished in this study. We calculated the relative risks (RRs) and 95% confidence intervals (CIs) for each of these outcomes by vitamin D status using binomial regression. We used Cox proportional hazards models to assess the association of vitamin D status with mortality in all patients and HIV disease progression from stage 3 to 4 in HIV-infected participants. We defined the end of follow-up as the date when HIV stage was last assessed.

We examined the association of vitamin D status with CD4, CD8, and CD3 T-cell counts, viral load (in HIV-infected participants), indicators of nutritional status (body mass index [BMI] and albumin concentrations), and hemoglobin concentrations using generalized estimating equations (GEEs). These models do not require that all patients have the same number of follow-up assessments or that the follow-up measurements be obtained at exactly the same time points. We assumed a standard normal distribution for repeated continuous end points (T cell subsets, $\log_{10}$ viral load, anthropometry, and albumin and hemoglobin concentrations) and estimated average differences during follow-up by vitamin D status. We used an exchangeable correlation structure to account for within-subject correlations and adjusted the models for the follow-up time when the measurements had been obtained and for the baseline values.

We analyzed the data for the entire period and for the first 8 months, coinciding with the expected end of TB treatment. Multivariate analyses adjusted for age, Karnofsky score, baseline hemoglobin concentrations, viral load, HIV status, CD4 T-cell counts, and micronutrient supplementation, unless otherwise specified in the results section or the tables. All analyses were performed using SAS software version 9.3 (SAS Institute Inc., Cary NC).

Results

Baseline 25-hydroxyvitamin D concentrations were available for 677 patients out of the original cohort of 887. Mean 25-hydroxyvitamin D concentration was 69.8 (±21.5) nmol/L [27.9 (±8.6) ng/mL] and its distribution is shown in Figure 1. The baseline

characteristics of these 677 patients by HIV status are presented in Table 1. 36% of the HIV-infected patients had CD4 T-cell counts below 200 cells/μL. The mean body mass index (BMI) was 19.1 ± 2.7 kg/m^2. The median follow-up time for HIV-uninfected patients was 52 months (inter-quartile range [IQR]: 47-57 months) and for HIV-infected patients was 30 months (IQR: 15-41 months).

The mean 25-hydroxyvitamin D concentrations were significantly different across season of blood draw in this cohort (p=0.004). Tanzania has four seasons: dry (January-February); long rains (March-June); dry (July-October); short rains (November-December). The boxplot of vitamin D's association with season of blood draw is presented in Figure 2. In subgroup analyses, this association was only observed among the HIV-uninfected patients and not the HIV-infected patients.

We examined the correlates of vitamin D insufficiency, defined as serum 25(OH)D concentrations below 75 nmol/L (75 nmol/L) in Supplemental Tables 1 (HIV-uninfected) and 2 (HIV-infected). All factors that had univariate associations with p<0.20 were included in a multivariate model; only the factors that had p<0.05 were retained in the final model. Among the HIV-uninfected subset, patients enrolled in the dry winter season between July and October were 50% more likely to have vitamin D insufficiency, compared to patients enrolled in the dry summer season between January and February (p for season=0.002). Similarly, the participants with the lowest height were more likely to have vitamin D insufficiency (p=0.01). Finally, greater expenditure on food per person per day was associated with a lower risk of having inadequate vitamin D status (Risk

Ratio [RR] per 1000 Tanzanian Shillings [approximately 1 US Dollar at the time of the study]: 0.76; 95% Confidence Interval [CI]: 0.59, 0.98). In the HIV-infected subset, patients with higher hemoglobin concentrations at baseline were less likely to have vitamin D insufficiency, with a 7% lower risk per 1 g/dL higher hemoglobin level (p=0.007). On the other hand, higher number of CD4 T-cells was associated with a higher risk of having inadequate vitamin D status (4% higher risk per 100 CD4 T-cells/µL; p=0.02).

There was no significant association of vitamin D status at TB treatment initiation with mortality or HIV disease progression in this cohort (Table 2 includes only HIV-infected participants as there were only 13 deaths in the HIV-uninfected subset). There was no association observed between vitamin D status and treatment failure one month after initiation of TB treatment (Table 3). However, patients with vitamin D insufficiency (<75 nmol/L) had a 66% higher risk of relapse after becoming culture-negative at one month after initiation of TB treatment (95% CI: 4%, 164%). This association was more pronounced in those who were not HIV-infected at enrollment in the study (RR: 2.33; 95% CI: 1.26, 4.29). In analysis with continuous vitamin D levels, each nmol/L increase was associated with a 1% lower risk of relapse during follow-up (p=0.04).

Vitamin D insufficiency was observed to have no association with CD4 T-cell counts during the entire follow-up in either the HIV-infected or the HIV-uninfected subsets (Table 4). However, vitamin D insufficiency was associated with greater CD4 T-cell

counts during the first eight months of follow-up in the HIV-infected patients (mean difference: 58; 95% CI: 13, 104).

In analysis among HIV-infected patients with continuous vitamin D levels, each nmol/L higher vitamin D concentration was associated with a decrease of 3 CD8 and 3 CD3 T-cells per μL. Vitamin D insufficiency was associated with an average of 85 higher CD8 T-cells/μL during follow-up (95% CI: 4, 165). Similar results were observed when we restricted the analyses to the first eight months of follow-up, the duration of TB treatment at the time of the study in Tanzania. No relationship was observed with mean viral loads during follow-up in the patients who were HIV-infected at the time of enrollment.

In analysis examining association of vitamin D status with nutritional parameters in the entire period of follow-up, no significant relationship was observed with BMI, albumin, or hemoglobin concentrations (Table 5). During the first eight months of follow-up, patients with Vitamin D Insufficiency experienced a decline in BMI (Mean: -0.21 kg/m^2; 95% CI: -0.39, -0.02), compared to patients with adequate vitamin D status. These results were more pronounced in HIV-uninfected patients (Mean: -0.34; 95% CI: -0.60, -0.09) and not significant in HIV-infected patients. HIV-infected patients with vitamin D insufficiency had increased albumin levels (Mean: 0.94; 95% CI: 0.55, 1.32) during the first eight months of follow-up compared to patients with adequate vitamin D status.

Discussion

In this study among 677 patients with tuberculosis in Tanzania, more than 61% of the participants had 25-hydroxyvitamin D concentrations below 75 nmol/L (75 nmol/L). 25-hydroxyvitamin D concentrations were associated with the season of blood draw, money spent on food per person per day, and height in HIV-uninfected participants and hemoglobin concentrations and CD4 T-cell counts among HIV-infected patients. Vitamin D insufficiency (<75 nmol/L) was not associated with mortality, HIV disease progression, or treatment failure during follow-up in the entire cohort. However, patients with vitamin D insufficiency had an increased risk of experiencing TB relapse during follow-up. Further, vitamin D insufficiency was associated with a decline in CD8 and CD3 T-cells in both the first eight months (the duration of TB treatment) and the entire period of follow-up. A similar relationship was observed with BMI in the first eight months of follow-up.

Our study was conducted in Dar es Salaam, the largest urban center in Tanzania, and just six degrees south of the Equator. The prevalence of vitamin D insufficiency (>61%) in this study is higher than the approximately 40% found in a previous study among TB patients in Mwanza, Tanzania [12] and in our studies among HIV-infected pregnant women (~85% of them had stage 1 HIV disease, unlike this study) in Dar es Salaam [13 14]. However, this prevalence is lower than what was observed in a cross-sectional study in South Africa, where 88% of HIV-uninfected and 97% of HIV-infected TB patients had vitamin D insufficiency. The mean vitamin D concentration in this study was 69.8 nmol/L, compared to 86.5 nmol/L in the study in Mwanza and 28.8-40 nmol/L in the South African study. One study from Thailand observed similar levels (69.0 nmol/L) in

TB patients [15]; Thailand is located at a similar distance from the Equator as Tanzania, though it is in the northern hemisphere. Similar to the study in South Africa, the vitamin D levels were lowest in our study in the dry winter season between July and October, though the differences were not as stark. For example, the mean vitamin D concentration in January to March in the South African study was 56.8 nmol/L and 30.8 nmol/L between July and September, whereas in our study, the concentrations were 74.8 nmol/L for January through February, and 66.3 nmol/L for July through October.

Vitamin D is synthesized in the skin through the action of ultraviolet light on 7-dehydrocholesterol. Fatty fish, such as salmon and sardines, are good sources of vitamin D in the diet but are not widely available everywhere and are usually expensive. Increasing urbanization and a tendency to spend most time indoors are major factors that contribute to the inability of the skin to synthesize adequate amounts of vitamin D [16-18]. Additionally, the TB disease itself and/or the HIV co-infection in the participants in this study are probably the primary reasons for restricted physical activity, lack of adequate exposure to sunlight, and consequent low concentrations of vitamin D.

Several other investigators have examined correlates of vitamin D status in TB patients. The study in Mwanza found that marital status, BMI, and serum transferrin receptor concentrations were correlated with vitamin D status. Though the first two were correlated with vitamin D status in our study in univariate analyses, neither remained significant in multivariate analyses. We didn't measure serum transferrin receptor in our study, though we did observe a correlation of vitamin D status with hemoglobin

concentrations among the HIV-infected subset. Another study in South Africa found that TB status (active disease *vs.* latent infection), month of sampling, and BMI were significantly correlated with vitamin D status in multivariate analyses [3]. All patients in our study had active disease, and we didn't observe a relationship with BMI in our analyses. The study in South Africa incorporated only those correlates associated with serum 25(OH)D concentration with $P < 0.05$ in univariate analysis in the multivariate model. This may have precluded selection of important covariates and confounders, if measured, and produced biased estimates and confidence intervals; increasing the nominal significance level to 20% or more, as used in this study [19] can eliminate most of this bias. Most other studies have been with smaller sample sizes and have examined a limited set of covariates, compared to the current study.

There was no association of vitamin D status with mortality or HIV disease progression in this cohort, unlike our previous studies among HIV-infected pregnant women [13 20] or HIV-infected adults [21 22] in Tanzania. The major difference is that in our earlier studies [13 20], a large majority (~85%) of the participants had stage 1 or asymptomatic HIV disease, whereas in this study, most of the individuals were already at stage 3 disease.

The association of vitamin D insufficiency with TB recurrence/relapse, primarily driven by the HIV-uninfected subset, is a novel finding in a longitudinal study and has important implications. Vitamin D deficiency has been linked to TB in several studies – a hypothesis perhaps initially generated by the observed seasonality of TB. *In vitro* and

animal studies indicate that 1,25-dihydroxyvitamin D_3, the most active form of vitamin D, may increase mycobacterial killing by macrophages but also limits host damage by decreasing the gamma-interferon production [23-28]. In perhaps the strongest evidence to date for a role of vitamin D in tuberculosis, a study by Liu *et al* [29] found that the antimycobacterial response in humans is dependent on adequate availability of vitamin D.

A few randomized trials of vitamin D supplementation in TB patients have been conducted in the past few years [4 5 30]. In a randomized trial that was conducted among 365 TB patients in Guinea-Bissau starting antituberculosis treatment, overall mortality was 15% (54 of 365) at 1 year of follow-up and similar in both arms [5]. Martineau and colleagues didn't find a difference in median time to sputum culture conversion with vitamin D supplementation of 2.5 mg vitamin D3 at enrollment, 14, 28, and 42 days after starting TB treatment in 126 adults with sputum smear-positive PTB [4]. A recent report by Coussens *et al* from a subset of the 126 adults included in the trial above stated that median time to sputum smear conversion in the intervention arm was significantly shorter than in the control arm (23 *vs.* 36 days; p=0.04) [30]. The lack of effect and concordance in most of these trials is probably due to the dose and dosing interval used. It is worth noting however, that large intermittent doses of vitamin D may result in supraphysiological concentrations in some cases, which may be more harmful than helpful in their effects on the immune system [31].

Vitamin D insufficiency also was associated with T-cell subset counts only among the HIV-infected patients in this cohort. We can only speculate as to the reasons for the significantly higher increase in CD4 T-cells observed in patients with vitamin D insufficiency at baseline. One potential explanation is that HIV-infected patients with vitamin D insufficiency may experience more uncontrolled immune reconstitution, leading to a greater increase in CD4 T-cell counts, on treatment of TB, compared to patients with adequate vitamin D status. This may also explain why this relationship is observed only in the first eight months of follow-up and not subsequently.

The results for CD8 and CD3 T-cells are consistent with our previous studies among HIV-infected women in Tanzania [13 14]. This could suggest a possible role of vitamin D in inflammation. Although, the conventional role of CD8 cells is as cytotoxic cells, they also are effector cells in inflammation [32]. The involvement of vitamin D in modulating CD8 cells is also indicated by the fact that CD8 cells express the highest concentration of vitamin D receptor of the immune cells [33]. Other studies also have found that vitamin D suppresses antigen-stimulated proinflammatory cytokine responses, which may help speed up resolution of inflammatory responses that can lead to increased risk of mortality among TB patients [30].

TB, once known as ‘consumption’, is associated with significant wasting and weight loss. The observation that better vitamin D status among HIV-uninfected patients is associated with a greater increase in BMI during follow-up is likely related to decreased risk of

relapse among these patients, as well as improvement in quality of life through mechanisms such as better metabolism that were not directly assessed in this study. The major strengths of this study include a large number of participants, more than half of whom were HIV-infected, comprehensive assessment of clinical, immunological, socio-demographic, and nutritional parameters, and a long duration of follow-up. On the other hand, the major limitation is the possibility of reverse causation and residual confounding. We have attempted to minimize this through rigorous analyses and adjusting for several potential confounders, including hemoglobin concentrations, HIV status, viral load, CD4 T-cells, and Karnofsky score, in most analyses. The study results are generalizable to most settings with a high TB burden and widely prevalent vitamin D insufficiency.

In summary, our study results indicate that adequate vitamin D status is associated with better clinical and nutritional parameters during follow-up in a cohort of TB patients in Tanzania. While randomized trials of vitamin D supplementation among TB patients are urgently warranted, it is also imperative to conduct dose-response studies to determine ideal dose and duration for the supplement.

Acknowledgments

We thank the patients, whose participation made this study possible. We are grateful to the study coordinator, Dorothy Mallya, and to the study physicians, research nurses, and laboratory and administrative staff at Muhimbili University of Health and Allied Sciences in Dar es Salaam, Tanzania, for their contributions to the project.

Financial support

National Institute of Allergy and Infectious Diseases, National Institutes of Health (grant U01AI045441 to support the study)

The authors report NO conflict of interest

Author Contributions

SM wrote the first draft of the manuscript and analyzed and interpreted the data; FMM, RJB, SA, WU, EV, and WWF were investigators of the parent trial and contributed to field activities and oversight; RJB also helped with the analysis and interpretation of the data; all authors participated in study design and contributed to the final manuscript. All authors have also read and approved the final manuscript.

References

1. WHO. Global Tuberculosis Control. Geneva: World Health Organization, 2011.

2. Chaisson RE, Martinson NA. Tuberculosis in Africa--combating an HIV-driven crisis. N Engl J Med 2008;**358**(11):1089-92 doi: 358/11/1089 [pii] 10.1056/NEJMp0800809[published Online First: Epub Date]|.

3. Martineau AR, Nhamoyebonde S, Oni T, et al. Reciprocal seasonal variation in vitamin D status and tuberculosis notifications in Cape Town, South Africa. Proc Natl Acad Sci U S A 2011;**108**(47):19013-7 doi: 10.1073/pnas.1111825108[published Online First: Epub Date]|.

4. Martineau AR, Timms PM, Bothamley GH, et al. High-dose vitamin D(3) during intensive-phase antimicrobial treatment of pulmonary tuberculosis: a double-blind randomised controlled trial. Lancet 2011;**377**(9761):242-50 doi: 10.1016/S0140-6736(10)61889-2[published Online First: Epub Date]|.

5. Wejse C, Gomes VF, Rabna P, et al. Vitamin D as supplementary treatment for tuberculosis: a double-blind, randomized, placebo-controlled trial. American Journal of Respiratory and Critical Care Medicine 2009;**179**(9):843-50 doi: 10.1164/rccm.200804-567OC[published Online First: Epub Date]|.

6. Villamor E, Mugusi F, Urassa W, et al. A Trial of the Effect of Micronutrient Supplementation on Treatment Outcome, T Cell Counts, Morbidity, and Mortality in Adults with Pulmonary Tuberculosis. J Infect Dis 2008;**197**(11):1499-505 doi: 10.1086/587846 10.1086/587846 [pii][published Online First: Epub Date]|.

7. Karnofsky DA, Abelmann WH, Craver LF, et al. The use of nitrogen mustards in the palliative treatment of cancer. Cancer 1948;**1**:634-56

8. Fawzi WW, Msamanga GI, Spiegelman D, et al. A randomized trial of multivitamin supplements and HIV disease progression and mortality. N Engl J Med 2004;**351**(1):23-32

9. Baum M, Cassetti L, Bonvehi P, et al. Inadequate dietary intake and altered nutrition status in early HIV-1 infection. Nutrition 1994;**10**(1):16-20

10. Lohman TG, Roche AF, Martorell R. *Anthropometric standardization reference manual*. Champaign, IL: Human Kinetics Books, 1988.

11. WHO. Interim proposal for a WHO staging system for HIV infection and disease. Wkly Epidemiol Rec 1990;**65**:221-24

12. Friis H, Range N, Pedersen ML, et al. Hypovitaminosis D is common among pulmonary tuberculosis patients in Tanzania but is not explained by the acute phase response. J Nutr 2008;**138**(12):2474-80 doi: 138/12/2474 [pii] 10.3945/jn.108.094979[published Online First: Epub Date]|.

13. Mehta S, Giovannucci E, Mugusi FM, et al. Vitamin D status of HIV-infected women and its association with HIV disease progression, anemia, and mortality. PLoS ONE 2010;**5**(1):e8770 doi: 10.1371/journal.pone.0008770[published Online First: Epub Date]|.

14. Mehta S, Spiegelman D, Aboud S, et al. Lipid-soluble vitamins A, D, and E in HIV-infected pregnant women in Tanzania. European journal of clinical nutrition 2010;**64**(8):808-17 doi: 10.1038/ejcn.2010.76[published Online First: Epub Date]|.

15. Davies PD, Church HA, Bovornkitti S, et al. Altered vitamin D homeostasis in tuberculosis. Intern Med (Thailand) 1988;**4**:45-47

16. Norman AW. Nutritional Aspects of Vitamin D. http://vitamind.ucr.edu/nutri.html. Accessed Oct 9, 2006. 1999. http://vitamind.ucr.edu/nutri.html (accessed Oct 9, 2006).

17. Utiger RD. The need for more vitamin D. N Engl J Med 1998;**338**(12):828-9

18. Holick MF. High prevalence of vitamin D inadequacy and implications for health. Mayo Clin Proc 2006;**81**(3):353-73

19. Greenland S. Modeling and variable selection in epidemiologic analysis. Am J Public Health 1989;**79**(3):340-9

20. Mehta S, Mugusi FM, Spiegelman D, et al. Vitamin D status and its association with morbidity including wasting and opportunistic illnesses in HIV-infected women in Tanzania. AIDS Patient Care STDS 2011;**25**(10):579-85 doi: 10.1089/apc.2011.0182[published Online First: Epub Date]|.

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. The Journal of infectious diseases 2013;**207**(3):378-85 doi: 10.1093/infdis/jis693[published Online First: Epub Date]|.

22. Sudfeld CR, Wang M, Aboud S, et al. Vitamin D and HIV progression among Tanzanian adults initiating antiretroviral therapy. PloS one 2012;**7**(6):e40036 doi: 10.1371/journal.pone.0040036[published Online First: Epub Date]|.

23. Thoma-Uszynski S, Stenger S, Takeuchi O, et al. Induction of direct antimicrobial activity through mammalian toll-like receptors. Science 2001;**291**(5508):1544-7

24. Waters WR, Palmer MV, Nonnecke BJ, et al. Mycobacterium bovis infection of vitamin D-deficient NOS2-/- mice. Microb Pathog 2004;**36**(1):11-7

25. Waters WR, Nonnecke BJ, Foote MR, et al. Mycobacterium bovis bacille Calmette-Guerin vaccination of cattle: activation of bovine CD4+ and gamma delta TCR+ cells and modulation by 1,25-dihydroxyvitamin D3. Tuberculosis (Edinb) 2003;**83**(5):287-97

26. Waters WR, Nonnecke BJ, Rahner TE, et al. Modulation of Mycobacterium bovis-specific responses of bovine peripheral blood mononuclear cells by 1,25-dihydroxyvitamin D(3). Clin Diagn Lab Immunol 2001;**8**(6):1204-12

27. Kawakami K, Teruya K, Tohyama M, et al. [A therapeutic trial of experimental tuberculosis with gamma-interferon in an immunocompromised mouse model]. Kekkaku 1994;**69**(10):607-13

28. McMurray DN, Bartow RA, Mintzer CL, et al. Micronutrient status and immune function in tuberculosis. Ann N Y Acad Sci 1990;**587**:59-69

29. Liu PT, Stenger S, Li H, et al. Toll-Like Receptor Triggering of a Vitamin D-Mediated Human Antimicrobial Response. Science 2006:1123933 doi: 10.1126/science.1123933[published Online First: Epub Date]|.

30. Coussens AK, Wilkinson RJ, Hanifa Y, et al. Vitamin D accelerates resolution of inflammatory responses during tuberculosis treatment. Proc Natl Acad Sci 2012:Epub

31. Martineau AR. Bolus-dose vitamin D and prevention of childhood pneumonia. Lancet 2012;**379**(9824):1373-5 doi: 10.1016/S0140-6736(12)60405-X[published Online First: Epub Date]|.

32. Babbe H, Roers A, Waisman A, et al. Clonal expansions of CD8(+) T cells dominate the T cell infiltrate in active multiple sclerosis lesions as shown by micromanipulation and single cell polymerase chain reaction. J Exp Med 2000;**192**(3):393-404

33. Veldman CM, Cantorna MT, DeLuca HF. Expression of 1,25-dihydroxyvitamin D(3) receptor in the immune system. Arch Biochem Biophys 2000;**374**(2):334-8 doi: 10.1006/abbi.1999.1605 S0003-9861(99)91605-3 [pii][published Online First: Epub Date]|.

Figure Legends

1. Distribution of 25-hydroxyvitamin D concentrations at baseline (nmol/L)

2. Distribution of 25-hydroxyvitamin D concentrations by season of blood draw;

   Season 1: Dry (January-February); Season 2: Long Rains (March-June); Season 3: Dry (July-October); Season 4: Short Rains (November-December)

Tables

Table 1	**Baseline Characteristics of the Study Population (n=677)**		
		HIV-infected (n=344)	**HIV-uninfected (n=333)**
Variable		**Mean (Standard Deviation)**	**Mean (Standard Deviation)**
Age, years		34.4 ± 8.6	30.2 ± 9.2
Money spent on food per person per day, Tanzanian Shillings*		587.3 ± 445.9	580.1 ± 684.2
Hemoglobin, g/dL		9.9 ± 1.8	11.1 ± 1.7
Albumin, g/dL		2.8 ± 1.0	3.2 ± 1.1
CD3 T-cell count, cells/μL		1228.0 ± 608.5	1195.9 ± 404.8
CD4 T-cell counts, cells/μL		327.2 ± 246.2	709.2 ± 250.8
CD8 T-cell counts, cells/μL		826.9 ± 447.5	427.5 ± 188.2
Log(10) Viral Load, copies/mL		4.6 ± 1.0	N/A
Body Mass Index, kg/m^2		19.4 ± 2.8	18.8 ± 2.5
Mid-Upper Arm Circumference, cm		23.4 ± 2.7	23.1 ± 2.7
Follow-up time, days		916.8 ± 507.4	1532.9 ± 331.4
		n (%)	***n (%)***
Vitamin D insufficiency (serum 25-hydroxyvitamin D <75 nmol/L)		218 (63.4%)	200 (60.1%)

Vitamin D deficiency (serum 25-hydroxyvitamin D <50 nmol/L)		55 (16.0%)	51 (15.3%)
Sex			
	Male	203 (59.0%)	257 (77.2%)
	Female	141 (41.0%)	76 (22.8%)
Center			
	Mwananyamala	79 (23.0%)	88 (26.4%)
	Temeke	102 (29.7%)	83 (24.9%)
	Tandale	83 (24.1%)	91 (27.3%)
	Mbgala	31 (9.0%)	70 (21.0%)
	Amana	49 (14.2%)	1 (.3%)
Karnofsky Score <70%		45 (13.1%)	29 (8.7%)
Education Group			
	None	29 (8.4%)	36 (10.8%)
	Low <5years	35 (10.2%)	31 (9.3%)
	Primary 5-8 years	238 (69.2%)	233 (70.0%)
	Secondary/University	42 (12.2%)	33 (9.9%)

Cohabits with a Partner		200 (58.1%)	168 (50.5%)
Assets at home			
	None	92 (26.9%)	108 (32.4%)
	One	89 (26.0%)	85 (25.5%)
	2-3	122 (35.7%)	114 (34.2%)
	4-5	39 (11.4%)	26 (7.8%)
WHO HIV Disease Stage			
	3	240 (90.9%)	N/A
	4	24 (9.1%)	
CD4 T-cell categories, cells/μL			
	0-199	97 (35.9%)	0 (.0%)
	200-499	116 (43.0%)	69 (22.9%)
	500+	57 (21.1%)	232 (77.1%)
WHO BMI Group, kg/m^2			
	<16	26 (7.7%)	33 (9.9%)
	16-16.99	37 (10.9%)	45 (13.6%)
	17-18.49	73 (21.5%)	88 (26.5%)
	18.5-19.99	79 (23.3%)	70 (21.1%)
	20-21.99	77 (22.7%)	69 (20.8%)

	22+	47 (13.9%)	27 (8.1%)

*1 US Dollar ≅ 1000 Tanzanian Shillings at the time of the study

Table 2	Vitamin D Status and Mortality and HIV Disease Progression in HIV-infected TB Patients				
		Univariate		Multivariate	
Outcome	*n/N (%)*	*RR (95% CI)*	*p-value*	*RR (95% CI)*	*p-value*
Mortality					
Vitamin D insufficiency (<75 nmol/L)	61/218 (28.0%)	0.73 (0.50, 1.08)	0.12	0.70 (0.47, 1.04)	0.08
Adequate Vitamin D	43/126 (34.1%)				
Vitamin D deficient (<50 nmol/L)	20/55 (36.4%)	1.34 (0.82, 2.18)	0.25	0.91 (0.55 1.50)	0.71
Not deficient	84/289 (29.1%)				
Continuous Vitamin D (nmol/L)		1.00 (0.99, 1.01)	0.49	1.01 (1.00, 1.02)	0.15
HIV Disease Progression					

	Vitamin D insufficiency (<75 nmol/L)	46/150 (30.7%)	1.10 (0.67, 1.82)	0.71		1.08 (0.64, 1.82)	0.78
	Adequate Vitamin D	23/90 (25.6%)	Reference			Reference	
	Vitamin D deficient (<50 nmol/L)	14/34 (41.2%)	1.91 (1.05, 3.44)	**0.03**		1.48 (0.78, 2.82)	0.23
	Not deficient	55/206 (26.7%)	Reference			Reference	
	Continuous Vitamin D (nmol/L)		0.99 (0.98, 1.01)	0.30		1. 00 (0.99, 1.01)	0.57
p-values obtained using Cox Proportional Hazards Regression; RR: Risk Ratio; 95% CI: 95% Confidence Interval							
Multivariate analyses adjusted for Age, Karnofsky Score, Baseline Hemoglobin, Viral Load, HIV Status, CD4 Counts, and Micronutrient Supplementation							

Table 3	Vitamin D Status and Treatment Outcomes in TB Patients					
		Univariate		Multivariate		
Outcome	*n/N (%)*	*RR (95% CI)*	*p-value*	*RR (95% CI)*	*p-value*	
Treatment Failure by 1 month post-treatment initiation						
Vitamin D insufficiency (<75 nmol/L)	58/298 (19.5%)	1.06 (0.72, 1.55)	0.77	1.02 (0.70, 1.49)	0.93	
Adequate Vitamin D	34/185 (18.4%)					
Vitamin D deficient (<50 nmol/L)	15/75 (20.0%)	1.06 (0.65, 1.74)	0.82	1.13 (0.69, 1.86)	0.63	
Not deficient	77/408 (18.9%)					
Continuous Vitamin D (nmol/L)		1.00 (0.99, 1.01)	0.49	1. 00 (0.99, 1.01)	0.50	
Any Relapse (relapse after 1 month post-treatment initiation if culture negative at 1 month)						
Vitamin D insufficiency	51/227 (22.5%)	1.56 (0.98, 2.48)	0.06	1.66 (1.04, 2.64)	**0.03**	

	(<75 nmol/L)							
	Adequate Vitamin D	21/146 (14.4%)						
	Vitamin D deficient (<50 nmol/L)	13/56 (23.2%)		1.25 (0.73, 2.12)	0.41		1.40 (0.82, 2.39)	0.21
	Not deficient	59/317 (18.6%)						
	Continuous Vitamin D (nmol/L)			0.99 (0.98, 1.00)	0.06		0.99 (0.98, 1.00)	**0.04**

p-values obtained using Binomial Regression; RR: Risk Ratio; 95% CI: 95% Confidence Interval

Multivariate analyses adjusted for Age, Karnofsky Score, Baseline Hemoglobin, Viral Load, HIV Status, CD4 Counts, and Micronutrient Supplementation

Table 4	Vitamin D Status and T-cell Counts (cells/µL) in TB Patients															
			CD4 T-cells					CD8 T-cells					CD3 T-cells			
Outcome			*Adequate vitamin D, mean (SD)*[a]	*Vitamin D insufficiency, mean difference (95% CI)*[b]	*Vitamin D insufficiency, adjusted mean difference (95% CI)*[c]	*p-value*		*Adequate vitamin D, mean (SD)*[a]	*Vitamin D insufficiency, mean difference (95% CI)*[b]	*Vitamin D insufficiency, adjusted mean difference (95% CI)*[c]	*p-value*		*Adequate vitamin D, mean (SD)*[a]	*Vitamin D insufficiency, mean difference (95% CI)*[b]	*Vitamin D insufficiency, adjusted mean difference (95% CI)*[c]	*p-value*

Entire follow-up: HIV-infected patients															
	Vitamin D insufficiency (<75 nmol/L)		300 (234)	17 (-23, 56)	21 (-18, 59)	0.29		902 (457)	88 (7, 169)	85 (4, 165)	**0.04**		1298 (635)	101 (-4, 206)	103 (-5, 212)	0.06
	Vitamin D deficient (<50 nmol/L)		333 (225)	21 (-34, 76)	30 (-26, 86)	0.29		957 (424)	105 (-9, 219)	114 (-6, 234)	0.06		1392 (595)	104 (-47, 255)	125 (-28, 279)	0.11
	Continuous Vitamin D (per nmol/L)			0 (-1, 1)	-1 (-1, 0)	0.26			-3 (-5, -1)	-3 (-5, -1)	**0.0004**			-3 (-5, -1)	-3 (-6, -1)	**0.01**
Entire follow-up: HIV-uninfected patients																
	Vitamin D		771	-2 (-	3 (-45,	0.9		508	-25 (-	-22 (-	0.2		1351	-37 (-	-28 (-	0.4

	insufficiency (<75 nmol/L)		(235)	49, 45)	51)	1		(209)	63, 14)	60, 17)	7		(400)	109, 35)	99, 44)	5
	Vitamin D deficient (<50 nmol/L)		781 (241)	-34 (-99, 30)	-34 (-101, 32)	0.31		500 (195)	-1 (-64, 62)	3 (-61, 67)	0.93		1354 (397)	-33 (-136, 71)	-28 (-134, 79)	0.61
	Continuous Vitamin D (per nmol/L)			0 (-1, 1)	0 (-1, 1)	0.97			0 (-1, 1)	0 (-1, 1)	0.83			0 (-1, 2)	0 (-2, 2)	0.90
First 8 months of follow-up: HIV-infected patients																
	Vitamin D insufficiency (<75 nmol/L)		316 (237)	54 (8, 100)	58 (13, 104)	**0.01**		868 (470)	132 (29, 235)	119 (15, 223)	**0.02**		1279 (670)	190 (42, 337)	179 (28, 331)	**0.02**

	Vitamin D deficient (<50 nmol/L)		372 (264)	36 (-25, 97)	41 (-20, 101)	0.19		963 (471)	63 (-77, 203)	75 (-72, 221)	0.32		1443 (689)	67 (-125, 259)	101 (-93, 295)	0.31
	Continuous Vitamin D (per nmol/L)			-1 (-2, 0)	-1 (-2, 0)	**0.01**			-4 (-6, -1)	-4 (-6, -1)	**0.002**			-4 (-7, -1)	-5 (-8, -2)	**0.003**
First 8 months of follow-up: HIV-uninfected patients															
	Vitamin D insufficiency (<75 nmol/L)		724 (243)	1 (-52, 53)	6 (-47, 59)	0.82		461 (232)	-22 (-63, 20)	-17 (-57, 22)	0.39		1248 (446)	-38 (-121, 46)	-27 (-106, 52)	0.50
	Vitamin D deficient		731	-7 (-	-7 (-	0.8		454	4 (-73,	5 (-71,	0.9		1247	14 (-	17 (-	0.8

	(<50 nmol/L)		(237)	95, 80)	96, 81)	7		(209)	81)	81)	0		(410)	124, 153)	123, 156)	2
	Continuous Vitamin D (per nmol/L)			0 (-2,1)	0 (-2,1)	0.54			0 (-1, 1)	0 (-1, 1)	0.73			0 (-2, 1)	0 (-2, 1)	0.57

[a] Data are the means (SD) of the average measurement during follow-up for each participant

[b] Data are the mean difference between the low and the adequate vitamin D group, as defined in Column B. The mean differences, 95% confidence intervals (CIs), and corresponding p-values were estimated from generalized estimating equations, after adjustment for baseline measurements, follow-up time, and treatment (micronutrients *vs.* placebo) group.

[c] Multivariate analyses additionally adjusted for Age, Karnofsky Score, and Baseline Hemoglobin

Table 5	Vitamin D Status and Nutritional Parameters in TB Patients													
	Body Mass Index (kg/m^2)					Albumin concentration (g/dL)					Hemoglobin concentration (g/dL)			
Outcome	*Adequate vitamin D, mean (SD)*[a]	*Vitamin D insufficiency, mean difference (95% CI)*[b]	*Vitamin D insufficiency, adjusted mean difference (95% CI)*[c]	*p-value*		*Adequate vitamin D, mean (SD)*[a]	*Vitamin D insufficiency, mean difference (95% CI)*[b]	*Vitamin D insufficiency, adjusted mean difference (95% CI)*[c]	*p-value*		*Adequate vitamin D, mean (SD)*[a]	*Vitamin D insufficiency, mean difference (95% CI)*[b]	*Vitamin D insufficiency, adjusted mean difference (95% CI)*[c]	*p-value*
Entire														

follow-up: All patients														
Vitamin D insufficiency (<75 nmol/L)	21.20 (2.80)	-0.06 (-0.30, 0.17)	-0.08 (-0.30, 0.14)	0.46		3.42 (0.74)	-0.05 (-0.14, 0.04)	0.00 (-0.08, 0.08)	0.97		12.65 (1.80)	-0.16 (-0.40, 0.08)	-0.18 (-0.41, 0.05)	0.12
Vitamin D deficient (<50 nmol/L)	21.23 (3.00)	-0.16 (-0.46, 0.14)	-0.14 (-0.44, 0.15)	0.34		3.42 (0.72)	-0.05 (-0.17, 0.07)	0.02 (-0.08, 0.13)	0.65		12.42 (1.87)	0.15 (-0.16, 0.45)	0.17 (-0.11, 0.45)	0.24

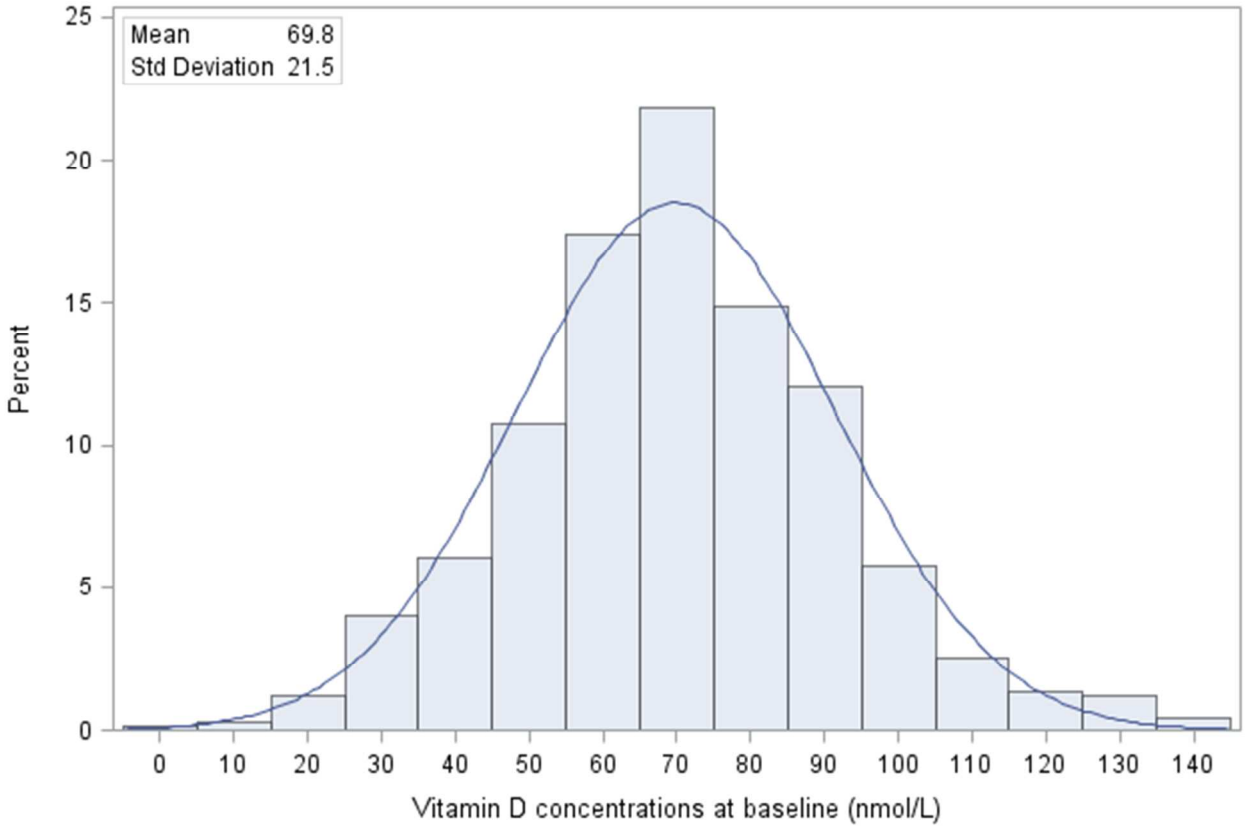
	Continuous Vitamin D (per nmol/L)	0.00 (0.00, 0.01)	0.00 (0.00, 0.01)	0.30			0.002 (0.00, 0.004)	0.000 (-0.002, 0.002)	0.90			0.00 (-0.01, 0.01)	0.00 (0.00, 0.01)	0.85
First 8 months of follow-up: All patients														
	Vitamin D insufficiency (<75 nmol/L)	20.96 (2.73)	-0.20 (-0.40, -0.01)	-0.21 (-0.39, -0.02)	**0.03**		3.42 (1.09)	-0.01 (-0.18, 0.16)	0.04 (-0.13, 0.21)	0.65		12.12 (1.85)	-0.01 (-0.28, 0.26)	-0.04 (-0.31, 0.23)	0.78

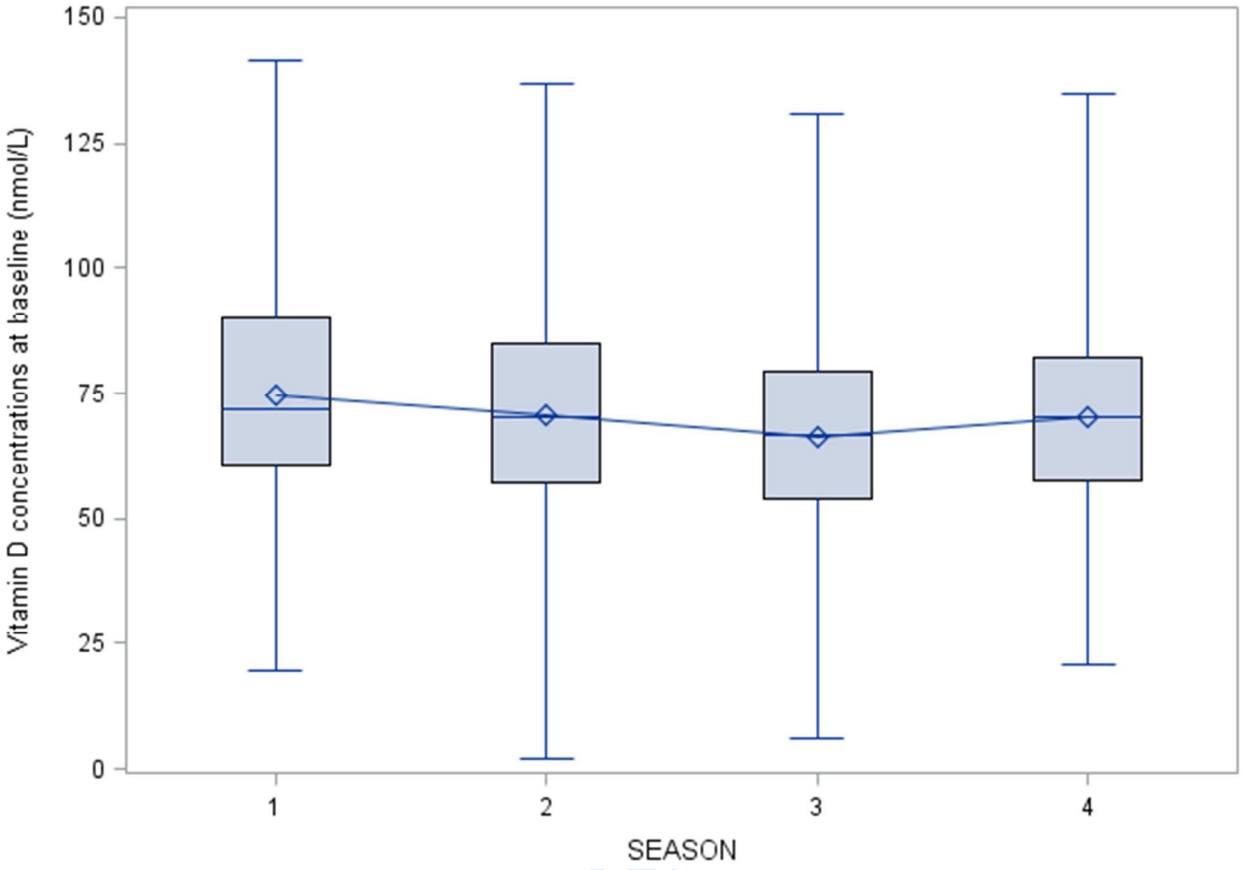
Vitamin D deficient (<50 nmol/L)	20.85 (2.84)	0.00 (-0.25, 0.25)	0.04 (-0.21, 0.29)	0.78	3.41 (1.08)	-0.11 (-0.32, 0.10)	-0.05 (-0.27, 0.17)	0.64	11.92 (1.99)	0.16 (-0.18, 0.50)	0.21 (-0.10, 0.53)	0.19
Continuous Vitamin D (per nmol/L)		0.00 (0.00, 0.01)	0.00 (0.00, 0.01)	0.38		0.000 (-0.003, 0.005)	0.000 (-0.004, 0.004)	0.87		0.00 (-0.01, 0.00)	0.00 (-0.01, 0.00)	0.57

[a] Data are the means (SD) of the average measurement during follow-up for each participant

[b] Data are the mean difference between the low and the adequate vitamin D group, as defined in Column B. The mean differences, 95% confidence intervals (CIs), and corresponding p-values were estimated from generalized estimating equations, after adjustment for baseline

measurements, follow-up time, and treatment (micronutrients *vs.* placebo) group.
[c] Multivariate analyses additionally adjusted for Age, Karnofsky Score, Baseline Hemoglobin, Viral Load, CD4 Count, and HIV Status; HIV status removed from the model where the results are stratified by HIV status. Viral Load also removed from the model in HIV-uninfected individuals.





Online Supporting Material

Supplemental Table 1	**Correlates of Vitamin D insufficiency (25(OH)D < 75 nmol/L) in HIV-uninfected TB patients**					
		Univariate (p<0.20)		**Multivariate (p<0.05)**		
Variable		**RR (95% CI)**	**p-value**	**RR (95% CI)**	**p-value**
Season			**0.002**		**0.002**
	1: Dry (Jan-Feb)	Ref.		Ref.	
	2: Long Rains (Mar-Jun)	1.07 (0.74, 1.53)		1.09 (0.76, 1.56)	
	3: Dry (Jul-Oct)	1.50 (1.07, 2.09)		1.50 (1.08, 2.08)	
	4: Short Rains (Nov-Dec)	1.29 (0.84, 1.96)		1.26 (0.84, 1.90)	
Sex	Female	1.19 (0.99, 1.43)	0.07		
Cohabits with a partner		0.89 (0.75, 1.06)	0.19		
Money spent per person per day on food, per 1000 Tanzanian Shillings*		0.76 (0.59, 0.98)	**0.04**	0.76 (0.59, 0.98)	**0.03**
Money spent per person per day on food quartiles			0.14		
	0:<250	1.36 (1.03, 1.80)			
	1:250-499	1.11 (0.82, 1.52)			
	2:500-750	1.13 (0.84, 1.53)			
	3:>750	Ref.			
AFB Culture positive at baseline		1.17 (0.94, 1.46)	0.16		
Number of colonies in AFB culture			0.16		
	1	Ref.			
	2	1.01 (0.66, 1.56)			
	3	0.95 (0.63, 1.44)			
	4	1.05 (0.70, 1.58)			

	5		1.27 (1.01, 1.59)				
Received TB treatment in the past 5 years			1.41 (0.97, 2.04)	0.07			
Hemoglobin, g/dL			0.92 (0.87, 0.97)	**0.004**			
CD4 T-cells, 100 cells/µL			1.03 (0.99, 1.07)	0.12			
CD3 T-cells, 100 cells/µL			1.02 (1.00, 1.04)	0.12			
Depressed >2 weeks, ever			1.17 (0.97, 1.42)	0.10			
Dysentery			0.23 (0.04, 1.41)	0.11			
Outpatient visit			1.14 (0.95, 1.38)	0.16			
Skin rash			0.73 (0.46, 1.16)	0.18			
Height quartiles, cm				**0.01**			**0.01**
	<158.1		Ref.			Ref.	
	158.1-164.0		1.08 (0.87, 1.32)			1.12 (0.93, 1.36)	
	164.1-169.5		0.76 (0.59, 0.98)			0.82 (0.64, 1.04)	
	169.6+		0.77 (0.60, 0.99)			0.81 (0.64, 1.03)	
Weight, kg			0.99 (0.97, 1.00)	**0.03**			
WHO BMI groups, kg/m^2				0.14			
	<16		1.14 (0.88, 1.48)				
	16-16.99		0.98 (0.74, 1.29)				
	17-18.49		Ref.				
	18.5-19.99		0.76 (0.57, 1.02)				
	20-21.99		0.89 (0.68, 1.15)				
	22+		1.11 (0.83, 1.48)				
Mid-Upper Arm Circumference (MUAC) <22 cm			1.27 (1.07, 1.51)	**0.01**			
Triceps Skinfold Thickness,			1.02 (1.00, 1.03)	0.09			

cm							
p-values obtained using Binomial Regression; RR: Risk Ratio; 95% CI: 95% Confidence Interval; *1 US Dollar ≅ 1000 Tanzanian Shillings at the time of the study							

Supplemental Table 2	**Correlates of Vitamin D insufficiency (serum 25(OH)D <75 nmol/L) in HIV-infected TB patients**					
		Univariate (p<0.20)		**Multivariate (p<0.05)**		
Variable		**RR (95% CI)**	**p-value**		**RR (95% CI)**	**p-value**
Age		0.99 (0.98, 1.00)	**0.01**			
Center			**0.03**			
	Mwananyamala	1.05 (0.86, 1.27)				
	Temeke	Ref.				
	Tandale	0.80 (0.63, 1.02)				
	Mbgala	0.67 (0.44, 1.01)				
	Amana	1.03 (0.82, 1.29)				
Cohabits with a partner		0.90 (0.77, 1.05)	0.19			
Assets at home			0.07			
	0:none	1.36 (1.03, 1.80)				
	1:one	0.88 (0.66, 1.16)				
	2:2-3	0.87 (0.67, 1.14)				
	3:4-5	Ref.				
Received TB treatment in the past 5 years		0.58 (0.31, 1.10)	0.10			
Hemoglobin, g/dL		0.95 (0.90, 1.00)	**0.04**		0.93 (0.89, 0.98)	**0.007**
Albumin, U/L		0.93 (0.86, 1.02)	0.13			
CD4 T-cells, 100 cells/µL		1.02 (0.99, 1.05)	0.12		1.04 (1.01, 1.07)	**0.02**
Depressed >2 weeks, ever		0.80 (0.61, 1.05)	0.11			
Hospitalization		1.30 (0.94, 1.80)	0.11			
Skin rash		1.32 (1.07, 1.64)	**0.01**			
Extrapulmonary TB		1.35 (0.93, 1.96)	0.11			

Height, cm			0.99 (0.98, 1.00)	0.08			
Weight, kg			0.99 (0.98, 1.00)	0.11			
WHO Body Mass Index (BMI) groups, kg/m^2				0.06			
	<16		0.81 (0.57, 1.16)				
	16-16.99		0.99 (0.76, 1.27)				
	17-18.49		Ref.				
	18.5-19.99		0.80 (0.63, 1.02)				
	20-21.99		0.75 (0.58, 0.96)				
	22+		1.05 (0.84, 1.30)				
Mid-Upper Arm Circumference (MUAC) quartiles, cm				**0.02**			
	<=21		Ref.				
	21.1-23.0		1.09 (0.90, 1.32)				
	23.1-24.9		0.81 (0.61, 1.08)				
	25+		0.82 (0.66, 1.03)				

p-values obtained using Binomial Regression; RR: Risk Ratio; 95% CI: 95% Confidence Interval

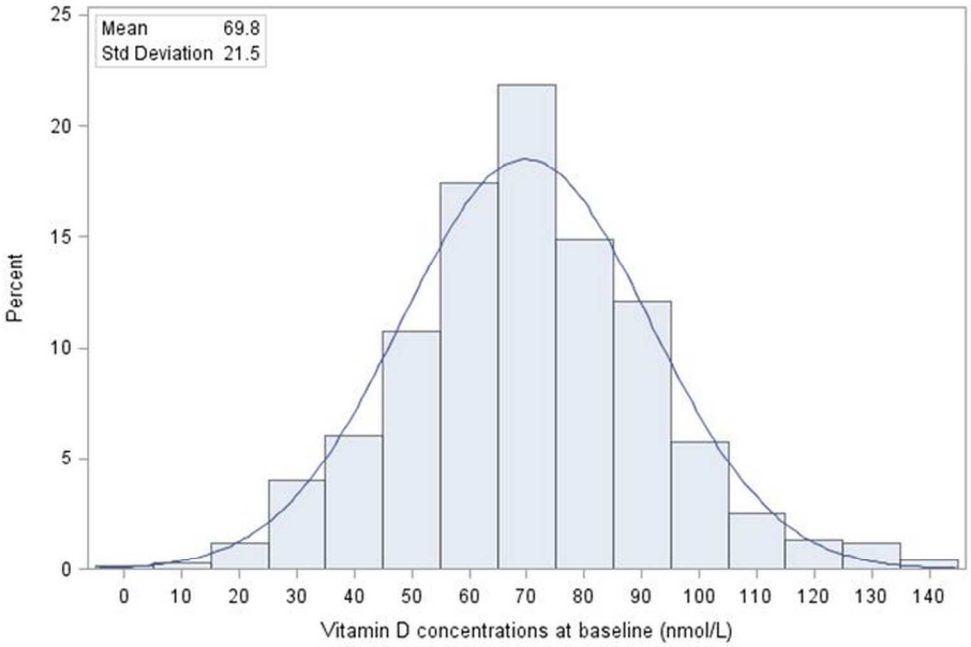
STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2-3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	5
Objectives	3	State specific objectives, including any prespecified hypotheses	6
Methods			
Study design	4	Present key elements of study design early in the paper	6-8
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6-8
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	6-8
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6, 9
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	9-10
Bias	9	Describe any efforts to address potential sources of bias	10
Study size	10	Explain how the study size was arrived at	10
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	10
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	9-10
		(*b*) Describe any methods used to examine subgroups and interactions	9-10
		(*c*) Explain how missing data were addressed	9-10
		(*d*) If applicable, explain how loss to follow-up was addressed	9-10
		(*e*) Describe any sensitivity analyses	NA
Results			

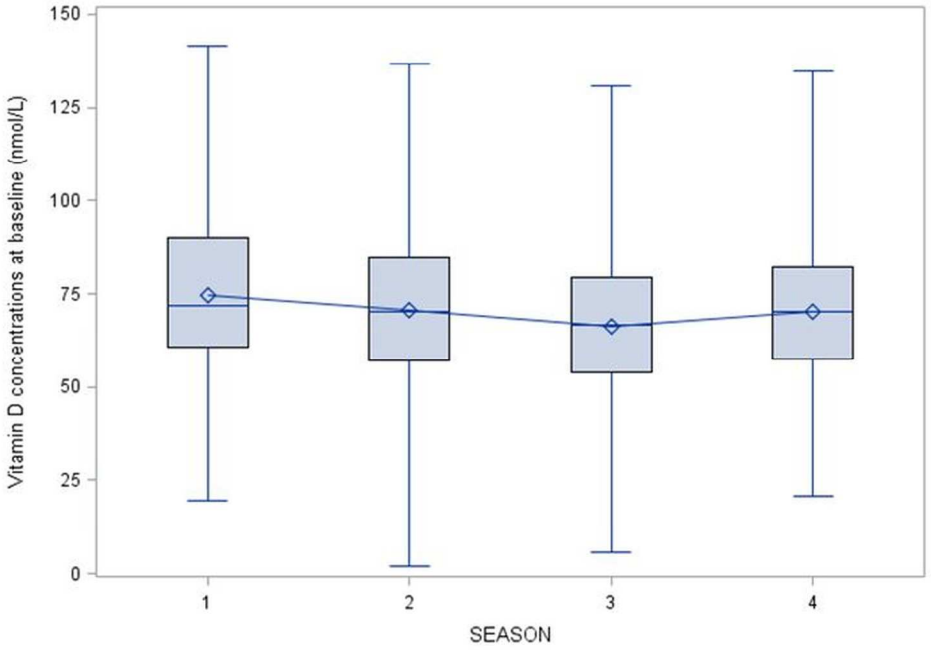
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	10, Tables
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	10-11
		(b) Indicate number of participants with missing data for each variable of interest	10
		(c) Summarise follow-up time (eg, average and total amount)	11
Outcome data	15*	Report numbers of outcome events or summary measures over time	Tables
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Tables
		(*b*) Report category boundaries when continuous variables were categorized	Tables
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	10-13
Discussion			
Key results	18	Summarise key results with reference to study objectives	13
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	14-19
Generalisability	21	Discuss the generalisability (external validity) of the study results	18-19
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	20

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.



131x90mm (300 x 300 DPI)



130x90mm (300 x 300 DPI)

Online Supporting Material

Supplemental Table 1	**Correlates of Vitamin D insufficiency (25(OH)D < 75 nmol/L) in HIV-uninfected TB patients**					
		Univariate (p<0.20)		**Multivariate (p<0.05)**		
Variable		**RR (95% CI)**	**p-value**	**RR (95% CI)**	**p-value**
Season			**0.002**		**0.002**
	1: Dry (Jan-Feb)	Ref.		Ref.	
	2: Long Rains (Mar-Jun)	1.07 (0.74, 1.53)		1.09 (0.76, 1.56)	
	3: Dry (Jul-Oct)	1.50 (1.07, 2.09)		1.50 (1.08, 2.08)	
	4: Short Rains (Nov-Dec)	1.29 (0.84, 1.96)		1.26 (0.84, 1.90)	
Sex	Female	1.19 (0.99, 1.43)	0.07		
Cohabits with a partner		0.89 (0.75, 1.06)	0.19		
Money spent per person per day on food, per 1000 Tanzanian Shillings*		0.76 (0.59, 0.98)	**0.04**	0.76 (0.59, 0.98)	**0.03**
Money spent per person per day on food quartiles			0.14		
	0:<250	1.36 (1.03, 1.80)			
	1:250-499	1.11 (0.82, 1.52)			
	2:500-750	1.13 (0.84, 1.53)			
	3:>750	Ref.			
AFB Culture positive at baseline		1.17 (0.94, 1.46)	0.16		
Number of colonies in AFB culture			0.16		
	1	Ref.			
	2	1.01 (0.66, 1.56)			
	3	0.95 (0.63, 1.44)			
	4	1.05 (0.70, 1.58)			

Online Supporting Material

	5		1.27 (1.01, 1.59)				
Received TB treatment in the past 5 years			1.41 (0.97, 2.04)	0.07			
Hemoglobin, g/dL			0.92 (0.87, 0.97)	**0.004**			
CD4 T-cells, 100 cells/µL			1.03 (0.99, 1.07)	0.12			
CD3 T-cells, 100 cells/µL			1.02 (1.00, 1.04)	0.12			
Depressed >2 weeks, ever			1.17 (0.97, 1.42)	0.10			
Dysentery			0.23 (0.04, 1.41)	0.11			
Outpatient visit			1.14 (0.95, 1.38)	0.16			
Skin rash			0.73 (0.46, 1.16)	0.18			
Height quartiles, cm				**0.01**			**0.01**
	<158.1		Ref.			Ref.	
	158.1-164.0		1.08 (0.87, 1.32)			1.12 (0.93, 1.36)	
	164.1-169.5		0.76 (0.59, 0.98)			0.82 (0.64, 1.04)	
	169.6+		0.77 (0.60, 0.99)			0.81 (0.64, 1.03)	
Weight, kg			0.99 (0.97, 1.00)	**0.03**			
WHO BMI groups, kg/m^2				0.14			
	<16		1.14 (0.88, 1.48)				
	16-16.99		0.98 (0.74, 1.29)				
	17-18.49		Ref.				
	18.5-19.99		0.76 (0.57, 1.02)				
	20-21.99		0.89 (0.68, 1.15)				
	22+		1.11 (0.83, 1.48)				
Mid-Upper Arm Circumference (MUAC) <22 cm			1.27 (1.07, 1.51)	**0.01**			
Triceps Skinfold Thickness,			1.02 (1.00, 1.03)	0.09			

cm							

p-values obtained using Binomial Regression; RR: Risk Ratio; 95% CI: 95% Confidence Interval; *1 US Dollar ≅ 1000 Tanzanian Shillings at the time of the study

Online Supporting Material

Supplemental Table 2	**Correlates of Vitamin D insufficiency (serum 25(OH)D <75 nmol/L) in HIV-infected TB patients**					
		Univariate (p<0.20)		**Multivariate (p<0.05)**		
Variable		**RR (95% CI)**	**p-value**		**RR (95% CI)**	**p-value**
Age		0.99 (0.98, 1.00)	**0.01**			
Center			**0.03**			
	Mwananyamala	1.05 (0.86, 1.27)				
	Temeke	Ref.				
	Tandale	0.80 (0.63, 1.02)				
	Mbgala	0.67 (0.44, 1.01)				
	Amana	1.03 (0.82, 1.29)				
Cohabits with a partner		0.90 (0.77, 1.05)	0.19			
Assets at home			0.07			
	0:none	1.36 (1.03, 1.80)				
	1:one	0.88 (0.66, 1.16)				
	2:2-3	0.87 (0.67, 1.14)				
	3:4-5	Ref.				
Received TB treatment in the past 5 years		0.58 (0.31, 1.10)	0.10			
Hemoglobin, g/dL		0.95 (0.90, 1.00)	**0.04**		0.93 (0.89, 0.98)	**0.007**
Albumin, U/L		0.93 (0.86, 1.02)	0.13			
CD4 T-cells, 100 cells/µL		1.02 (0.99, 1.05)	0.12		1.04 (1.01, 1.07)	**0.02**
Depressed >2 weeks, ever		0.80 (0.61, 1.05)	0.11			
Hospitalization		1.30 (0.94, 1.80)	0.11			
Skin rash		1.32 (1.07, 1.64)	**0.01**			
Extrapulmonary TB		1.35 (0.93, 1.96)	0.11			

Height, cm			0.99 (0.98, 1.00)	0.08			
Weight, kg			0.99 (0.98, 1.00)	0.11			
WHO Body Mass Index (BMI) groups, kg/m^2				0.06			
	<16		0.81 (0.57, 1.16)				
	16-16.99		0.99 (0.76, 1.27)				
	17-18.49		Ref.				
	18.5-19.99		0.80 (0.63, 1.02)				
	20-21.99		0.75 (0.58, 0.96)				
	22+		1.05 (0.84, 1.30)				
Mid-Upper Arm Circumference (MUAC) quartiles, cm				**0.02**			
	<=21		Ref.				
	21.1-23.0		1.09 (0.90, 1.32)				
	23.1-24.9		0.81 (0.61, 1.08)				
	25+		0.82 (0.66, 1.03)				

p-values obtained using Binomial Regression; RR: Risk Ratio; 95% CI: 95% Confidence Interval