Vitamin D status and risk of incident tuberculosis disease: a systematic review and individual participant data meta-analysis

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ABSTRACT

Background: Few studies have evaluated the association between pre-existing vitamin D deficiency (VDD) and incident TB. We assessed the impact of baseline vitamin D on TB risk.

Methods: We assessed the association between baseline vitamin D and incident TB in a prospective cohort of 6751 household contacts of TB patients in Peru. We also conducted a one-stage individual participant data meta-analysis searching PubMed and Embase for studies of vitamin D and TB until December 31, 2017. We included studies that assessed vitamin D before TB diagnosis. We defined VDD as 25–(OH)D <50 nmol/L, insufficiency as 50–75 nmol/L and sufficiency as >75nmol/L. We estimated the association between vitamin D and incident TB using conditional logistic regression in the Peru cohort and generalized linear mixed models in the meta-analysis.

Findings: In Peru, baseline VDD was associated with a statistically insignificant increase in incident TB (aOR 1·70, 95% CI 0·84–3·46; p=0·14). We identified seven studies for the meta-analysis and analyzed 3544 participants. Individuals with VDD and very low vitamin D (<25nmol/L) had increased TB risk (aOR 1·48, 95% CI 1·04–2·10; *p*=0·03 and aOR 2·08, 95% CI 0·88–4·92; *p* trend=0·02 respectively). Among HIV-positive patients, VDD and very low vitamin D conferred a 2-fold (aOR 2.18, 95% CI 1·22–3·90; p=0·01) and 4-fold (aOR 4·28, 95% CI 0·85–21·44; p trend=0·01) increased risk of TB respectively.

Interpretation: Our findings suggest vitamin D predicts TB risk in a dose-dependent manner and vitamin D supplementation may play a role in TB prevention.

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Research in Context

Evidence before this study

Numerous studies have found lower serum vitamin D levels among patients with active TB disease compared to healthy controls. However, research has not clarified whether low vitamin D increases TB risk or whether TB disease leads to decreased vitamin D levels. We conducted PubMed and Medline searches for all studies available through December 31, 2017 on the association between vitamin D status and TB disease. We included the following keywords: “vitamin D,” “vitamin D deficiency,” “hypovitaminosis D,” “25-hydroxyvitamin D,” “1,25-dihydroxyvitamin D,” “vitamin D2,” “vitamin D3,” “ergocalciferol,” “cholecalciferol,” and “tuberculosis.” We found only seven studies had prospectively evaluated the impact of baseline vitamin D levels on risk of progression to TB disease.

We report here the results of a case control study nested within a large prospective longitudinal cohort study of household contacts of TB cases and the results of an individual participant data (IPD) meta-analysis of available evidence on the association between vitamin D levels and incident TB disease.

Added value of this study

We demonstrated that low vitamin D levels predicts risk of future progression to TB disease in a dose-dependent manner.

Implications of all the available evidence

These findings suggest the possibility that vitamin D supplementation among individuals at high risk for developing TB disease might play a role in TB prevention efforts.

Introduction

The global burden of tuberculosis (TB) remains high with approximately one-fourth to one-third of world's population infected with *Mycobacterium tuberculosis*, and the World Health Organization (WHO) estimates 10 million people developed TB disease in 2017.[1] Concurrently, vitamin D deficiency (VDD) is a widespread problem, with reported adult prevalence of VDD ranging from 10% to 80% worldwide.[2,3] Vitamin D is an important regulator of the immune system,[4] and *in vitro* studies have elucidated some of the mechanisms by which vitamin D influences TB disease pathogenesis.[5,6] The discovery that vitamin D activates cathelicidin-mediated killing of ingested mycobacteria in macrophages[5] has focused attention on the possibility that low vitamin D levels may contribute to TB disease progression.

Numerous observational studies have also documented lower serum vitamin D levels among TB patients compared to healthy controls, and prior meta-analyses investigating the association between vitamin D and TB have concluded that low vitamin D increases TB disease risk.[7–10] However, most studies were cross-sectional studies and assessed vitamin D status after the diagnosis of active TB disease, rather than the impact of pre-existing vitamin D levels on the risk of progression to TB disease. Given TB disease can induce profound metabolic abnormalities, it remains unclear whether vitamin D deficiency increases TB disease risk or whether TB disease leads to decreased serum vitamin D levels. Furthermore, prior studies evaluating the association between vitamin D and TB disease have used different cutoffs to categorize vitamin D levels or define vitamin D deficiency.[7–10] Hence, it is challenging to determine whether there is a vitamin D threshold below which individuals are at risk of TB disease.

Here, we address the association of vitamin D levels on the risk of TB progression in two ways. We first report results of a case control analysis nested in a prospective cohort study of household contacts of TB patients that we conducted in Lima, Peru. We next pool these data with those from other published prospective studies of vitamin D status and TB risk to conduct an individual participant data (IPD) meta-analysis synthesizing available evidence on the association between vitamin D levels and incident TB disease.

Methods

Lima Cohort Study

Ethics Statement

The study was approved by the Institutional Review Board of Harvard School of Public Health and the Research Ethics Committee of the National Institute of Health of Peru. All study participants or guardians provided written informed consent.

Study Setting and Population

Details of the study design and methods are described elsewhere[11,12] and presented in the appendix (p 1). We enrolled a prospective cohort of household contacts (HHCs) of index TB patients in Lima, Peru between September 2009 and August 2012. We screened HHCs for pulmonary and extra-pulmonary TB disease at baseline and 2, 6, and 12 months after enrollment. We offered HIV testing and invited HHCs to provide a baseline blood sample. We classified HHCs as having incident secondary TB disease if they were diagnosed at least 15 days after index case enrollment and co-prevalent TB disease if they were diagnosed earlier.

We defined "cases" as HIV-negative HHCs with blood samples who developed incident secondary TB disease within one year of follow-up. For each case, we randomly selected four controls from among HHCs who were not diagnosed with TB disease, matching on gender and age by year.

At the end of follow up, we measured levels of total 25–(OH)D and retinol from stored blood samples.

Statistical Analysis

We defined vitamin D deficiency as serum 25–(OH)D < 50 nmol/L, insufficiency as 50–75 nmol/L and sufficiency as > 75nmol/L.[13] We used univariate and multivariate conditional logistic regression models to evaluate the association between baseline VDD and risk of TB disease. Because we had previously observed that vitamin A deficiency increases TB risk in this cohort,[11] we also adjusted for vitamin A levels.

In sensitivity analyses, we restricted the analysis to HHCs diagnosed with incident TB at least 60 days after index patient enrollment and their matched controls. We also separately evaluated patients with microbiologically confirmed TB and their matched controls.

Systematic Review and IPD Meta-analysis

Search Strategy and Data Sources

We conducted the systematic review and meta-analysis according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRIMSA) guidelines.[14,15] All studies included in the IPD meta-analysis received relevant institutional or country-specific ethics approval, and participants provided written or oral informed consent.

We searched PubMed (https://www.ncbi.nlm.nih.gov/pubmed) and EMBASE (https://www.embase.com) for all available studies up to December 31, 2017 on the association between vitamin D and incident TB disease. Supplementary table 1 provides details of our search strategy (appendix p 3).

Study Eligibility and Inclusion Criteria

We placed no restrictions on language. We included all prospective longitudinal studies of human participants at risk for TB if the study measured vitamin D levels in baseline blood samples obtained prior to a diagnosis of TB disease. Studies were included if they confirmed TB diagnosis by microbiological criteria or specified their clinical criteria for ascertaining TB disease and if they collected data on age and gender. We considered any clinical criteria for TB disease based on physician assessment, imaging studies or national guidelines and any laboratory method for assessing vitamin D levels. We did not require studies to control for possible confounders. We also considered reports from conference abstracts. We excluded the following: case reports; animal or in vitro studies; case-control or cross-sectional studies that measured vitamin D levels after TB diagnosis; studies that did not report vitamin D levels; studies of other diseases or non TB-related outcomes; studies of vitamin D and TB treatment outcomes or TB infection or TB immune reconstitution inflammatory syndrome (IRIS); reviews; meta-analyses; letters; editorials; and protocols.

Data Collection and Data Items

For each eligible study, two reviewers (OA and SC) independently performed full text reviews and extracted the following information: first author's last name, year of publication, study design and study

aim, country of study, calendar years of study, length of follow up, number of incident TB cases, total number of subjects analyzed, criteria for diagnosing TB disease, laboratory method of vitamin D assay, method of categorizing vitamin D, assessment of HIV status, and covariates in multivariate analyses. Discrepancies were resolved by consensus.

We contacted all authors of the eligible studies identified from the systematic review and requested individual participant data. Lead investigators from all studies agreed to provide data, which were de-identified prior to transfer via email. We requested all available data on possible confounders of the association between vitamin D and TB disease including: age, gender, HIV status, weight, height, BMI, isoniazid preventive treatment, baseline tuberculin skin test result, TB disease history, and comorbid diseases. We also requested baseline vitamin D levels, incident TB disease status during study follow up, time from enrollment to TB diagnosis, and index case smear status if applicable. We reviewed data for consistency with published data and contacted authors for clarifications or missing information as needed.

Statistical Analysis

We conducted a one-stage IPD meta-analysis combining data from eligible studies and the Lima cohort study. We used the unified criteria described above to define VDD (25–(OH)D < 50 nmol/L) and insufficiency (25–(OH)D 50–75 nmol/L). We further defined “very low” vitamin D levels as less than 25nmol/L. We considered datasets from each single-country study and from each country within a multi-country study as independent data sources. We used generalized linear mixed univariate and multivariate models to evaluate the association between baseline vitamin D deficiency and risk of incident TB, including an indicator for each independent dataset as a random effect to account for within-study correlation. Multivariate models were adjusted for age, gender, BMI, and HIV. Given sparse data on other variables, we did not adjust for other potential confounders. We also separately evaluated the association between very low vitamin D levels, compared to sufficient levels, and TB disease risk. To determine whether the effect of vitamin D on incident TB differed by HIV status, we conducted a stratified analysis. In a sensitivity analysis, we restricted the main analysis to incident TB cases diagnosed at least 60 days after enrollment.

The IPD meta-analysis was conducted using the R package "lme4”.[16]

Role of the funding source

The funding sources had no role in the study design, data collection, analysis, and interpretation, writing of the manuscript, and in the decision to submit for publication. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Lima Cohort Study

Among 6751 HIV-negative household contacts with baseline blood samples, 258 developed TB disease, 66 within 15 days of enrollment and 192 thereafter. Among these 192 secondary TB cases, 152 (79.1%) were microbiologically confirmed, and viable blood samples were available for analysis for 180 (93.8%) at the end of follow-up. Table 1 lists baseline characteristics of the incident cases and their matched controls. The median levels of vitamin D at baseline were similar among cases (53·9 nmol/L; IQR 42·7 – 64·0 nmol/L) and controls (54·7 nmol/L; IQR 44·5 – 67·1 nmol/L; p 0·32) [Table 2]. Median vitamin D level during spring/summer (57·2 nmol/L; IQR 46·1–69·0 nmol/L) was higher than during winter (49·6 nmol/L; IQR 40·5–59·9 nmol/L; p < 0·001).

In the univariate analysis, household contacts with baseline vitamin D deficiency had a 54% increased risk of incident TB disease compared to those with sufficient levels though this was not statistically significant (95% CI 0·88 – 2·71; p 0·13) [Table 3]. Vitamin D insufficiency was associated with a smaller increase in risk of TB disease that was not significant (OR 1·23; 95% CI 0·72 – 2·08; p 0·45). After adjusting for BMI categories, socioeconomic status, heavy alcohol consumption, tobacco use, isoniazid preventive therapy, TB history, comorbid disease, self-reported DM, index patient smear status, and season of sample collection, we found baseline vitamin D deficiency and insufficiency remained associated with an increased risk of incident TB disease that was not statistically significant (aOR 1·70; 95% CI 0·84 – 3·46; p 0·14 and aOR 1·25; 95% CI 0·66 – 2.40; p 0·50 respectively) [Table 3]. When we further adjusted for vitamin A deficiency, we continued to find a non-significant increase in risk of TB disease among HHCs with vitamin D deficiency and sufficiency (aOR 1·60; 95% CI 0·76 – 3·39; p 0·22 and aOR 1·21; 95% CI 0·62 – 2·39; p 0·58 respectively).

Our conclusions did not differ from results of the main analysis when we restricted our analyses to cases (and matched controls) diagnosed at least 60 days after index patient enrollment or to microbiologically confirmed TB cases (Table 4).

Systematic Review and IPD Meta-analysis

We identified 2689 citations from the PubMed and EMBASE searches. After screening titles and abstracts, we excluded 2678 articles because they were reviews, meta-analyses, letters, editorials or protocols (n = 1212), case reports (n = 515), studies of other diseases or other outcomes (n = 331), animal or *in vitro* studies (n = 247), case-control or cross-sectional studies that assessed vitamin D levels after TB disease diagnosis (n = 159), studies that did not measure vitamin D levels (n = 144), studies of TB treatment outcomes (n = 63), and studies of TB infection (n = 7) [Figure 1]. We reviewed full texts of the remaining 11 articles[17–27] and further excluded three studies that assessed outcomes of TB-IRIS[24–26] and one study of TB infection with seasonality of TB.[27] Table 5 provides information about the seven eligible published studies[17–23] identified from the systematic review.

We obtained individual patient data from all eligible studies. One study provided patient data from a multi-site evaluation conducted in nine countries.[23] Six of the seven studies were prospective cohort or case-cohort studies[17,18,20–23] while one study was a nested case-control study.[19] The final combined dataset with our Lima cohort study included 3544 participants from 13 countries: Brazil, The Gambia, Haiti, India, Malawi, Pakistan, Peru, South Africa, Spain, Tanzania, Thailand, US, and Zimbabwe. We analyzed a total of 456 incident TB cases. The median time to TB diagnosis from enrollment was 152·0 days (IQR 44·0 – 342·0 days). Table 6 lists the baseline characteristics of all patients analyzed. The majority of the participants (82·1%) were adults ≥ 18 years of age. HIV status was unknown for 629 (17·7%) patients while 1711 (48·3%) were HIV positive. The median baseline level of 25–OH Vitamin D was 65·0nmol/L (IQR 48·8 – 83·5 nmol/L). The prevalence of vitamin D deficiency at baseline was 26·2% and of "very low" vitamin D levels was 4·8%. Most of the participants with very low vitamin D levels were from studies conducted in India (17·5%),[19] Spain (25·2%)[17] and Pakistan (35·7%).[22]

In the univariate analysis, baseline vitamin D deficiency was associated with a 49% increased risk of progression to TB disease (OR 1·49; 95% CI 1·07 – 2·07; p 0·02) [Table 7]. Vitamin D insufficiency also increased the risk of incident TB in a dose-dependent manner (OR 1·26; 95% CI 0·95 – 1·66; p trend 0·02). Both vitamin D deficiency and insufficiency remained associated with an increased risk of TB disease after we adjusted for age, gender, BMI and HIV status (aOR for deficiency: 1·48; 95% CI 1·04 – 2·10; p 0·03 and aOR for insufficiency: 1·33; 95% CI 1·00 – 1.77; p 0·05).

When we stratified by HIV status, we found that HIV-positive individuals with vitamin D deficiency were twice as likely to develop TB disease compared to those with normal levels (aOR 2·18; 95% CI 1·22 – 3·90; p 0·01) while HIV-negative participants were only 20% more likely to develop disease (aOR 1·20; 95% CI 0·75 – 1·94; p 0·44) [Table 8, p for interaction = 0·17].

Compared to those with sufficient levels, participant with very low vitamin D levels were twice as likely to develop TB (aOR 2·08; 95% CI 0·88 – 4·92; p trend 0·02) [Table 9]. Among HIV-positive individuals, very low vitamin D levels conferred a 4·28-fold increased risk of TB (95% CI 0·85 – 21·44; p trend 0·01). In contrast, very low vitamin D among HIV-negative individuals was associated with a 58% increase in TB disease risk that was not statistically significant (aOR 1·58; 95% CI 0·57 – 4·43; p trend 0·45) [Table 9, p for interaction 0·17].

When we separately considered incident cases diagnosed at least 60 days after enrollment, vitamin D insufficiency remained associated with increased risk of TB disease (aOR 1·42; 95% CI 1·04 – 1·94; p 0·03) while vitamin D deficiency was no longer associated with incident TB (aOR 1·02; 95% CI 0·67 – 1·57; p 0·91).

Discussion

The two analyses presented here provide consistent support for a modest dose-dependent effect of vitamin D on future progression of TB disease across multiple studies conducted in diverse contexts. In the IPD, the association of low vitamin D levels with increased TB disease risk is especially strong among HIV-positive individuals whose risk of developing TB was more than four-fold higher for those with very low levels of vitamin D.

Our findings in these human studies support the role of vitamin D in TB disease that has been inferred from more fundamental research which has shown that vitamin D is an important regulator of innate immunity.[4] *In vitro* studies have enumerated multiple mechanisms by which VDD may influence pathogenesis of TB infection or disease. Vitamin D is implicated in the activation of cathelicidin-mediated killing of ingested mycobacteria,[5,30] induction of IFN-γ-mediated activity in macrophages,[6] induction of reactive oxygen and nitrogen species,[31] stimulation of phagolysosome fusion in infected macrophages,[32] and inhibition of matrix metalloproteinases involved in the pathogenesis of cavitary pulmonary TB.[33]

In addition to its impact on immunity, vitamin D status has been linked to human metabolic phenotypes that may be involved in the pathogenesis of TB. *In vitro* studies have demonstrated various ways by which vitamin D promotes insulin sensitivity,[34] and animal models have shown VDD impairs insulin secretion in pancreatic beta cells.[34,35] Numerous observational studies have also found an inverse association between vitamin D levels and incident type 2 diabetes mellitus (DM).[34] Given DM is a well described risk factor for TB disease,[36,37] vitamin D deficiency may also contribute to increased TB risk through its role in modifying risk of diabetes.

Two other lines of evidence point to a possible association between vitamin D and TB. First, multiple studies have reported an association between specific vitamin D receptor (VDR) polymorphisms and TB risk.[8,38] Although it is not clear that the functional effect of these polymorphisms recapitulates the impact of low vitamin D levels, several studies show that the impact of VDR variants is stronger in the presence of VDD.[18,38,39] Secondly, TB incidence varies with season and peaks in summer months when vitamin D levels are highest. Some observers have postulated that low levels of sunshine, and hence vitamin D, in

winter contribute to an increase in TB infection followed by a rise in TB disease incidence after a six-month lag.[27,40–42]

We also note previous studies have reported that low vitamin A is a strong predictor of incident TB disease.[23,43] In a previous analysis of the Lima cohort, we found vitamin A deficiency conferred a 10-fold increase in TB disease risk,[11] and here we show that adjustment for vitamin A modestly attenuates the impact of vitamin D. Similarly, Tenforde et al. also reported that adjusting for vitamin A levels attenuated the effect of vitamin D on TB disease risk.[23] This raises the possibility that vitamin D levels correlate with other micronutrients implicated in the pathogenesis of TB and these micronutrients may be potent mediators of increased TB risk.

Although we did not detect a statistically significant interaction between vitamin D levels and HIV status, our findings raise the possibility that the effect of low vitamin D on TB risk may be more pronounced among HIV-positive patients. Studies have shown that among HIV-infected individuals, VDD is associated with deleterious immune activation,[44] lower CD4 counts,[44,45] higher viral loads,[44] and accelerated HIV disease progression.[44,46] Thus, VDD may exacerbate existing immune dysregulation in HIV infection to further increase TB risk or low vitamin D levels may reflect severity of HIV-related immunosuppression. In vitro studies have also demonstrated vitamin D restricts mycobacterial growth in the presence of HIV infection.[47] A clinical trial is currently underway to evaluate the efficacy of vitamin D supplementation in preventing incident TB among adults with HIV in Tanzania;[52] the results may help clarify role of vitamin D in HIV-associated TB disease. We also plan to measure inflammatory markers in the Peru cohort to explore association between VDD and immune dysregulation.

We considered possible explanations for why we did not detect a significant association between VDD and TB risk in the Peru cohort, despite its relatively large size. First, since TB incidence is highest in summer[41, 42] and HHCs were recruited when the index case was diagnosed, they are more likely to have been recruited and assessed when their vitamin D levels were highest. If levels later fell and this fall precipitated TB progression, this would not have been detected. Secondly, VDR variants are heterogeneously distributed in different populations and may modify the effect of vitamin D on TB risk. We did not measure VDR variants in Peru and are therefore unable to assess the prevalence of different VDR genotypes in this cohort. The Peru study is also limited by the relatively short (one year) period of follow-up and the fact that it was only powered to detect a three-fold or greater difference in TB incidence among people with VDD.

Our IPD meta-analysis also has some important limitations. Firstly, many possible confounding covariates were not measured across all studies. Therefore, we were unable to account for all possible factors such as baseline infection status, other micronutrient levels and comorbidities that might be associated with both VDD and TB risk. Secondly, although we only examined prospective studies of incident TB disease, the included studies were all observational, and we cannot exclude the possibility that participants had early, undiagnosed TB at baseline that lowered vitamin D levels. Although we addressed this by conducting a sensitivity analysis excluding incident TB cases diagnosed less than 60 days after enrollment, the smaller number of incident cases diagnosed after 60 days reduced the power to detect a statistically significant association. We also cannot exclude the possibility of publication bias if studies with non-significant findings on the link between vitamin D and incident TB have remained unpublished. We did not construct a funnel plot to assess publication bias because we analyzed fewer than ten studies, which used different methods to categorize vitamin D levels and therefore provided effect estimates are not directly comparable. During our systematic review, we attempted to address this by considering data reported from meeting abstracts and none met our inclusion criteria.

In conclusion, in our meta-analysis of prospective studies, we found that vitamin D deficiency was associated with increased risk of incident TB disease. This finding suggests that vitamin D status is a predictor of incident TB disease; however, randomized control trials are needed to determine whether vitamin D supplementation can reduce the risk of developing TB disease. Future research should also elucidate the mechanisms by which low vitamin D influences TB risk.

Author Contributions

Lima Cohort Study: MBM and MCB led the study design. LL oversaw data collection and management with JTG, RC, ZZ, CC, and RY. RC managed laboratory efforts. MBM supervised data analysis and interpretation in conjunction with OA, C-CH, and MFF.

IPD Meta-Analysis: MBM conceived the study and supervised data extraction, pooling, analysis, and interpretation with C-CH, OA and SC. SA, AA, JB, RB, GD, WWF, DG, MG, BG, AG, NG, RH, JI, NTI, JJ, AK, VM, NM, GM, FMM, OAO, JP, FR, MR, SAS, CRS, MWT, TOT undertook individual studies and patient enrollment.

OA and MBM wrote the first draft of the manuscript, and all authors contributed to manuscript revision.

Declaration of Interests

Authors declare no competing interests.

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Figure Legends

Figure 1. Flow diagram for selection of studies for the individual participant data (IPD) meta-analysis

Table 1. Baseline characteristics of participants in Lima cohort study.

	Cases (N = 180) n (%)	**N* (Cases)**	**Controls (N = 709) n (%)**	**N* (Controls)**	**Non-Matched household contacts (N = 5796) n (%)**	**N* (Household contacts)**
Age Categories in years						
< 10	4 (2·2)		16 (2·3)		244 (4·2)	
10 to 19	50 (27·8)		200 (28·2)		1024 (17·7)	
≥ 20	126 (70·0)		493 (69·5)		4528 (78·1)	
Male	94 (52·2)		366 (51·6)		2413 (41·6)	
BMI Categories†		179		706		5745
Underweight	8 (4·5)		6 (0·9)		64 (1·1)	
Overweight	45 (25·1)		299 (42·4)		2881 (50·2)	
Normal	126 (70·4)		401 (56·8)		2800 (48·7)	
Socioeconomic Status‡		171		698		5607
Lowest Tertile	77 (45·0)		228 (32·7)		1847 (32·9)	
Middle Tertile	66 (38·6)		326 (46·7)		2565 (45·8)	
Highest Tertile	28 (16·4)		144 (20·6)		1195 (21·3)	
Heavy Alcohol Use§	14 (8·1)	174	64 (9·3)	692	444 (7·9)	5632
Current Smoking	13 (7·4)	176	78 (11·2)	699	488 (8·5)	5711
Self-Reported Diabetes	6 (3·4)	179	11 (1·6)	701	135 (2·4)	5739
Comorbid Disease¶	37 (20·6)		175 (24·7)		1380 (23·8)	5795
Isoniazid Preventive Therapy	7 (3·9)		108 (15·2)		713 (12·3)	5790
BCG scar	159 (88·3)		628 (88·6)		5133 (88·6)	5795
History of TB	34 (18·9)		55 (7·8)	708	545 (9·4)	5782
TB Infection at baseline	145 (82·4)	176	281 (41·1)	683	2768 (49·0)	5649
Index Patient Characteristics						
Smear Positive	156 (86·7)		486 (68·7)	707	4150 (71·6)	5793
Cavitary Disease	54 (30·2)	179	175 (25·1)	697	1424 (24·9)	5722

Abbreviation: BCG, Bacillus Calmette–Guérin; BMI, body mass index

* Total number of subjects with data for corresponding variable.

† We classified adults ≥ 20 years as underweight (BMI < 18·5 kg/m^2), normal weight (BMI 18·5–< 25 kg/m^2), and overweight (BMI ≥ 25 kg/m^2). For children and adolescents < 20 years, we used World Health Organization (WHO) age and gender-specific BMI z-scores tables to classify those with BMI z-score < –2 as underweight and those with z-score > 2 as overweight.

‡ We used principal components analysis of housing asset (housing type, number of rooms, water supply, sanitation facilities, lighting, composition of exterior walls and floor and roof materials) weighted by household size to compute a socioeconomic status score.

§ Self-reported consumption of ≥ 40g or ≥ 3 alcoholic drinks daily.

¶ Heart disease, high blood pressure, asthma, kidney disease, use of steroids or chemotherapy or immunosuppressant, any other self-reported chronic illness

Table 2. Baseline levels of vitamin D among cases and controls in Lima cohort study.

	Cases (N = 180) median (IQR) or n (%)	**Control (N = 709) median (IQR) or n (%)**	**p value***
25–OH Vitamin D (nmol/L)	53·9 (42·7 – 64·0)	54·7 (44·5 – 67·1)	0·32
Vitamin D Deficient (< 50 nmol/L)	76 (42·2)	259 (36·5)	0·13
Vitamin D Insufficient (50 – 75 nmol/L)	84 (46·7)	348 (49·1)	0·45
Vitamin D Sufficient (> 75 nmol/L)	20 (11·1)	102 (14·4)	1·00

* Univariate p values adjusted for matching factors (age and sex)

Table 3. Association between vitamin D levels and risk of TB disease among household contacts of TB patients in Lima cohort study.

	Cases/Controls	Univariate OR (95% CI) N = 889	p value	Multivariate OR* (95% CI) N = 822	p value
Vitamin D Deficient (< 50 nmol/L)	76/259	1·54 (0·88 – 2·71)	0·13	1·70 (0·84 – 3·46)	0·14
Vitamin D Insufficient (50 – 75 nmol/L)	84/348	1·23 (0·72 – 2·08)	0·45	1·25 (0·66 – 2·40)	0·50
Vitamin D Sufficient (> 75 nmol/L)	20/102	1·00		1·00	

***** Adjusted for matching factors (age and sex), body mass index (BMI) categories, socioeconomic status, heavy alcohol consumption, tobacco use, isoniazid preventive therapy, TB history, comorbid disease, self-reported DM, index patient smear status, and season of sample collection.

Table 4. Vitamin D levels and risk of TB disease for TB diagnosed at least 60 days after index case enrollment and microbiologically confirmed TB in Lima cohort study.

	Cases/Controls	Multivariate OR* (95% CI)	P value
TB diagnosed ≥ 60 days			
Vitamin D Deficient (< 50 nmol/L)	26/87	1·66 (0·62 – 4·45)	0·31
Vitamin D Insufficient (50 – 75 nmol/L)	50/211	1·13 (0·49 – 2·60)	0·77
Vitamin D Sufficient (> 75 nmol/L)	15/65	1·00	
Microbiologically Confirmed TB Disease			
Vitamin D Deficient (< 50 nmol/L)	60/209	1·78 (0·79 – 4·03)	0·17
Vitamin D Insufficient (50 – 75 nmol/L)	72/284	1·46 (0.68 – 3·12)	0·33
Vitamin D Sufficient (> 75 nmol/L)	15/84	1·00	

* Adjusted for matching factors (age and sex), body mass index (BMI) categories, socioeconomic status, heavy alcohol consumption, tobacco use, isoniazid preventive therapy, TB history, comorbid disease, self-reported DM, index patient smear status, and season of sample collection.

Table 5. Summary of published studies included in the individual participant data (IPD) meta- analysis.

Study (reference)	Country	Study design and study population	Method of measuring vitamin D	Length of follow up	TB cases/ Total Number of participants	TB disease definition	Adjusted effect estimate (95% CI)
Arnedo-Pena et al., 2015 (17)	Spain	Prospective cohort study of household and community contacts of TB cases	Electro-chemiluminescence (ECLIA) and Chemiluminescence immunoassays (CLIA)	Mean 1·6 years (± 0·9 years)	3/523	Smear or culture positive	aHR for continuous vitamin D: 0·88 (0·80 – 0·97)
Gupta et al., 2016 (18)	South Africa	Prospective case-cohort study of HIV positive and HIV exposed infants	Immunoassay	192 weeks	100/366	2004 South African NTP criteria for definite, probable or possible TB (28)	aHR for vitamin D < 80nmol/L: 1·76 (1·01 – 3·05)
Mave et al., 2015 (19)	India	Nested case-control study of HIV positive breastfeeding mothers	Radioimmunoassay	12 months	33/120	Culture confirmed OR Probable TB: 1) smear positive; and 2) histological and clinical features suggestive of TB and response to anti-TB treatment	aOR for vitamin D < 50 nmol/L: 1·57 (0·49 – 4·98)
Owolabi et al., 2016 (20)	The Gambia	Prospective cohort study of household contacts of TB cases	ELISA	24 months	12/139	smear/culture positive	Adjusted linear regression estimate for continuous vitamin D: 3·65 (0·59 – 6·71)
Sudfeld et al., 2013 (21)	Tanzania	Prospective cohort study of HIV positive patients initiating anti-retroviral therapy (ART)	High Performance Liquid Chromatography	Median 20·6 months (IQR 8·4 – 33·8 months)	50/1092	smear positive or CXR	aHR for vitamin D < 50 nmol: 2·89 (1·31 – 7·41)
Talat et al., 2010 (22)	Pakistan	Prospective cohort study of household contacts of TB cases	ELISA	4 years	8/109	smear positive or CXR	aHR for 1-log decrement in continuous vitamin D: 5·1 (1·2 – 21·3)
Tenforde et al., 2017 (23)	Brazil, Haiti, India, Malawi, Peru, South Africa, Thailand, US, Zimbabwe	Prospective case-cohort study of HIV positive patients initiating ART	Immunoassay	96 weeks	70/306	AIDS Clinical Trial Group (ACTG) Criteria for confirmed, probable or clinical TB (29, Appendix 60)	aHR for vitamin D < 50 nmol: 3·66 (1·16 – 11·51)

Table 6. Baseline demographic and clinical characteristics of participants in the individual-participant data (IPD) meta-analysis (N = 3544).

	n (%) or median (IQR)
Male	1520 (42·9)
Age categories in years	
< 5	376 (10·6)
5 to 17	257 (7·3)
≥ 18	2911 (82·1)
HIV	
Positive	1711 (48·3)
Negative	1204 (34·0)
Unknown	629 (17·7)
Body Mass Index (BMI) Categories*	
Underweight	433 (12·2)
Normal	1858 (52·4)
Overweight	873 (24·6)
Unknown	380 (10·7)
Isoniazid Preventive Therapy	
Yes	366 (10·3)
No	2504 (70·7)
Unknown	674 (19·0)
Baseline Tuberculin Skin Test	
Positive (≥ 10 mm)	750 (21·2)
Negative	965 (27·2)
Unknown	1829 (51·6)
History of TB	
Yes	558 (15·7)
No	2447 (69·1)
Unknown	539 (15·2)
Comorbid Disease	
Yes	1224 (34·5)
No	1181 (33·3)
Unknown	1139 (32·1)
Index smear status among studies of household contacts of TB cases†	
Positive	1155 (69·6)
Negative	364 (21·9)
Unknown	141 (8·5)
Antiretroviral therapy use among HIV positive‡	
Yes	1588 (92·8)
No	121 (7·1)
Unknown	2 (0·1)
Baseline CD4 count among HIV positive (cell/μL)§	167 (82 – 272)

25–OH Vitamin D (nmol/L)	65·0 (48·8 – 83·5)
Vitamin D Deficient (< 50 nmol/L)	930 (26·2)
Vitamin D Insufficient (50 – 75 nmol/L)	1357 (38·3)
Vitamin D Sufficient (> 75 nmol/L)	1257 (35·5)

* We classified adults ≥ 20 years as underweight (BMI < 18·5 kg/m^2), normal weight (BMI 18·5–< 25 kg/m^2), and overweight (BMI ≥ 25 kg/m^2). For children and adolescents < 20 years, we used World Health Organization (WHO) age and gender-specific BMI z-scores tables to classify those with BMI z-score < –2 as underweight and those with z-score > 2 as overweight.
† N = 1660
‡ N = 1711
§ N = 1353

Table 7: Association between selected baseline characteristics and risk of incident TB disease in the individual participant data (IPD) meta-analysis.

	Univariate OR* **(95% CI)** **N = 3544**	**P value**	**Multivariate OR†** **(95% CI)** **N = 2769**	**P value**
Body mass index (BMI) Categories‡				
Underweight	1·44 (1·02 – 2·04)	0·04	1·37 (0·96 – 1·96)	0·08
Normal	Ref		Ref	
Overweight	0·43 (0·32 – 0·58)	<0·001	0·43 (0·32 – 0·59)	<0·001
HIV positive§	1·44 (0·92 – 2·25)	0·11	1·23 (0·77 – 1·97)	0·38
Vitamin D Deficient (< 50 nmol/L)	1·49 (1·07 – 2·07)	0·02	1·48 (1·04 – 2·10)	0·03
Vitamin D Insufficient (50 – 75 nmol/L)	1·26 (0·95 – 1·66)	0·11	1·33 (1·00 – 1·77)	0·05
Vitamin D Sufficient (> 75 nmol/L)	1·00		1·00	
		P trend = 0·02		P trend = 0·03

* Adjusted for age and gender due to presence of age and gender matched case control study in the combined dataset

† Adjusted for age, gender, BMI categories and HIV status

‡ N = 3164

§ N = 2915

Table 8: Vitamin D deficiency and risk of incident TB disease stratified by HIV status in the individual participant data (IPD) meta-analysis.*

	HIV Positive		**HIV Negative**	
	Multivariate OR† (95% CI) N = 1576	**P value**	**Multivariate OR† (95% CI) N = 1193**	**P value**
Vitamin D Deficient (< 50 nmol/L)	2·18 (1·22 – 3·90)	0·01	1·20 (0·75 – 1·94)	0·44
Vitamin D Insufficient (50 – 75 nmol/L)	1·32 (0·90 – 1·94)	0·16	1·19 (0·76 – 1·85)	0·45
Vitamin D Sufficient (> 75 nmol/L)	1·00		1·00	
		P trend = 0·01		P trend = 0·50
Body Mass Index (BMI) Categories				
Normal	Ref		Ref	
Underweight	1·02 (0·68 – 1·54)	0·92	3·98 (1·79 – 8·85)	0·001
Overweight	0·41 (0·21 – 0·78)	0·01	0·45 (0·32 – 0·63)	<0·001

* Excluded datasets from Arnedo-Pena et al. (ref 17) and Talat et al. (ref 22) due to lack of information on HIV status

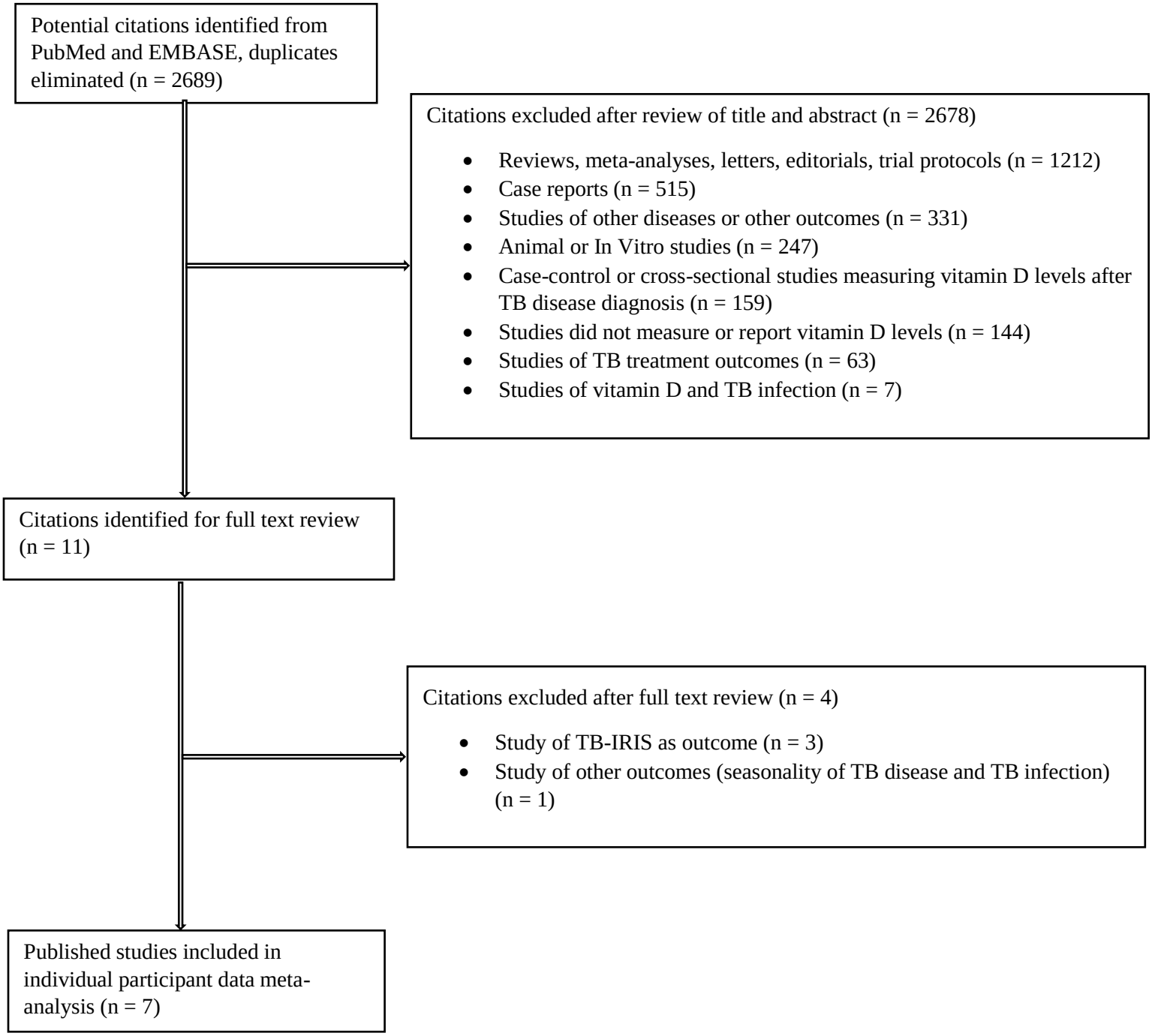
† Adjusted for age, gender and body mass index (BMI) categories

Table 9: Very low vitamin D levels (< 25 nmol/L) and risk of incident TB disease stratified by HIV status in the individual participant data (IPD) meta-analysis.

	Multivariate OR (95% CI)	P value
All Participants (N = 2769)*		
Vitamin D < 25 nmol/L	2·08 (0·88 – 4·92)	0·10
Vitamin D 25 – < 50 nmol/L	1·44 (1·01 – 2·06)	0·04
Vitamin D Insufficient (50 – 75 nmol/L)	1·33 (1·00 – 1·77)	0·05
Vitamin D Sufficient (> 75 nmol/L)	1.00	
		P trend = 0·02
HIV Positive (N = 1576)†		
Vitamin D < 25 nmol/L	4.28 (0·85 – 21·44)	0·08
Vitamin D 25 – < 50 nmol/L	2·06 (1·13 – 3·76)	0·03
Vitamin D Insufficient (50 – 75 nmol/L)	1·32 (0·90 – 1·94)	0·15
Vitamin D Sufficient (> 75 nmol/L)	1·00	
		P trend = 0·01
HIV Negative (N = 1193)†		
Vitamin D < 25 nmol/L	1·58 (0·57 – 4·43)	0·38
Vitamin D 25 – < 50 nmol/L	1·18 (0·73 – 1·91)	0·50
Vitamin D Insufficient (50 – 75 nmol/L)	1·19 (0·76 – 1·84)	0·45
Vitamin D Sufficient (> 75 nmol/L)	1·00	
		P trend = 0·45

* Model adjusted for age, gender, body mass index (BMI) categories and HIV status

† Model adjusted for age, gender, body mass index (BMI) categories


Potential citations identified from PubMed and EMBASE, duplicates eliminated (n = 2689)
Citations excluded after review of title and abstract (n = 2678)
Reviews, meta-analyses, letters, editorials, trial protocols (n = 1212)
Case reports (n = 515)
Studies of other diseases or other outcomes (n = 331)
Animal or In Vitro studies (n = 247)
Case-control or cross-sectional studies measuring vitamin D levels after TB disease diagnosis (n = 159)
Studies did not measure or report vitamin D levels (n = 144)
Studies of TB treatment outcomes (n = 63)
Studies of vitamin D and TB infection (n = 7)
Citations identified for full text review (n = 11)
Citations excluded after full text review (n = 4)
Study of TB-IRIS as outcome (n = 3)
Study of other outcomes (seasonality of TB disease and TB infection) (n = 1)
Published studies included in individual participant data meta-analysis (n = 7)