Avicenna J Clin Microb Infec. 2017 February; 4(1):e36889. doi: 10.17795/ajcmi-36889.

Published online 2016 September 4. **Research Article**

Tuberculosis and Vitamin D Status Among the Contacts of Pulmonary Tuberculosis Patients

Alberto Arnedo-Pena,[1,2,*] Jose Vicente Juan-Cerdan,[3] Maria Angeles Romeu-Garcia,[1] Daniel Garcia-Ferrer,[3] Jesus Iborra-Millet,[3] Jose Antonio Ferrero-Vega,[3] Juan Bautista Bellido-Blasco,[1,2] Noemi Meseguer-Ferrer,[1] and Francisco Pardo-Serrano[4]

[1]Epidemiology Division, Public Health Centre Castellon, Spain
[2]CIBER of Epidemiology and Public Health (CIBERESP), Barcelona, Spain
[3]Biochemical Laboratory, Hospital General, Castellon, Spain
[4]Microbiology Laboratory, Hospital General, Castellon, Spain

[*]*Corresponding author*: Alberto Arnedo-Pena, Epidemiology Division, Public Health Centre Castellon, Spain. Tel: +34-964399607, Fax: +34-064399645, E-mail: arnedo_alb@gva.es

Received 2016 February 03; **Revised** 2016 August 02; **Accepted** 2016 August 28.

Abstract

Background: Serum vitamin D (VitD) status is associated with active tuberculosis (TB) and TB infection conversion (TBIC).
Objectives: The objective of the present study was to quantify the risks of TB (latent, conversion, disease) in accordance with VitD status and other variables among the contacts of pulmonary TB patients.
Methods: From 2009 to 2012, a cohort of the contacts of pulmonary TB patients was studied to rule out and prevent TB in Castellon (Spain). The exams performed included a tuberculin skin test (TST), a QuantiFERON Gold in-tube test® (QFTGIT), blood and radiographic tests, and an initial measurement of serum VitD status. Contacts who were initially without active TB were followed up through 2015. Multinomial logistic regression (MLR) analyses were carried out.
Results: From a total of 572 contacts of pulmonary TB patients with VitD status measurement, 523 completed the follow-up (participation rate 91.4%). Among them, five groups could be established: 3 new cases of pulmonary TB (0.6%), 27 cases of TBIC (5.2%), 116 cases of latent TB infection (LTBI) (22.2%), 125 uninfected TB contacts with only one TST or QFTGIT measurement (23.9%), and 252 uninfected TB contacts with two TST or QFTGIT measurements (48.2%). The comparison of these five groups revealed several significant differences, including age, whether they were foreign-born, place of residence, social class, high exposure to an index case with sputum acid-fast bacilli (AFB), and VitD status. The MLR analysis for all groups, with the group of uninfected TB contacts with two TST or QFTGIT measurements as a reference, estimated that only two of these factors were significantly associated with TB in three or more groups; these factors were VitD status and high exposure and a sputum AFB-positive index case. VitD status was a protector against pulmonary TB with a relative risk (RR) of 0.86 (95% confidence interval [CI] 0.74 - 0.99) and against TBIC (R = 0.95; 95% CI 0.91 - 0.99), while it was not associated with LTBI (RR = 0.99; 95% CI 0.97-1.01). Only 34.0% of the contacts had sufficient VitD levels ($\geq$ 30 ng/mL).
Conclusions: Our results suggest that a sufficient VitD level could be a protective factor against TBIC and active TB.

Keywords: Tuberculosis, Vitamin D, Cohort Study, Interferon-Gamma Release Assays, Tuberculin Test, Contacts Tuberculosis

1. Background

Serum vitamin D (VitD) status is considered a factor in the development of tuberculosis (TB) (1). Low levels of VitD have been associated with an increase of pulmonary TB (2, 3), and two prospective cohort studies of the contacts of pulmonary TB patients that considered VitD status have found this relationship (4, 5). However, VitD supplements added to the standard treatments of pulmonary TB in several clinical trials have not been able to significantly eliminate *Mycobacterium tuberculosis* (*M. tuberculosis*) from the sputum of patients to date, though several clinical aspects have been improved (6-12). Since VitD supplements have been indicated to prevent TB infection (13), the first preventive clinical trial on Mongolian children was carried out with close to significant results (14).

A cohort study of the contacts of pulmonary TB patients may allow for the quantification of the association of VitD status with TB disease, latent TB infection (LTBI), and TB infection conversion (TBIC), as we have already studied in recent publications (15-17). In addition, an analysis of this cohort as a whole may be useful to obtain measurements of the association of VitD with other potential factors.

2. Objectives

The objective of this study was to quantify the risks of TB (latent, conversion, disease) in accordance with vitamin D status and other variables among contacts of pulmonary TB patients.

3. Methods

3.1. Study Design

Recruitment of participants included in the prospective cohort study of the contacts of pulmonary TB patients took place during 2009 - 2012. Follow-up was carried out by the epidemiology division of the public health center of castellon and the preventive medicine department of the hospital La Plana of Vila-real and finished in 2015. The Vila-real area is situated at a latitude of 39° north on the Mediterranean coast of Spain, with a population of 470,000 inhabitants. The epidemiology division implemented the follow-up of the cohort through the permanent TB register and the population information system of the Valencian community health care system.

3.2. Tuberculosis Diagnostic Tests

In order to rule out and prevent TB, contacts were examined and a tuberculin skin test (TST) with 2 tuberculin units equivalent to 5IU of purified protein derivative RT-23 (PPD) from the serologic institute of Copenhagen and/or a QuantiFERON-TB Gold in-tube test (QFTGIT® Cellestis Limited), which is an interferon-gamma release assay, were administered. In addition to these tests, clinical, analytical, and thorax radiographic exams were performed. Moreover, all participants were interviewed about TB risk factors. A more detailed methodology has been reported in previous publications (15, 16). For the participants, chemoprophylaxis against TB was recommended, taking into account exposure, age, clinical conditions, and the characteristics of the index TB case. The following definitions were used (18-20):

3.2.1. New Pulmonary TB

Diagnosis of TB based on laboratory tests and thorax radiographic exams, including determination of acid-fast bacilli (AFB) in sputum through smear microscopy and sputum culture of *M. tuberculosis*. The laboratory study was carried out by the microbiology laboratory of the hospital general of Castellon (HGC) and the hospital La Plana of Vila-real.

3.2.2. LTBI

A positive TST with induration size $\geq$ 5 mm when not vaccinated against Bacilli Calmette-Guerin (BCG) or $\geq$ 15 mm when BCG-vaccinated or a QFTGIT $\geq$ 0.35 IU/mL. In addition, clinical and radiographic exams were negative.

3.2.3. TBIC

Eight to ten weeks after the initial examination with negative TST, an increase of induration of 5 mm when non-BCG-vaccinated or 10 mm when BCG-vaccinated. For the QFTGIT analysis, the definition was the change from a negative < 35 IU/mL test to a positive test $\geq$ 35 IU/mL. Booster TST was indicated for contacts > 54 years old and/or BCG-vaccinated when the initial TST was negative 7 days after the first TST. In addition, clinical and radiographic exams were negative.

On the basis of these definitions and the follow-up, five groups were studied: 1, new pulmonary TB patients; 2, TBIC patients; 3,LTBI patients; 4, uninfected TB contacts when only one TST or QFTGIT measurement was required (the date of study was greater than two months after exposure to the TB index case) or contacts without second TST or QFTGIT measurements, and 5, uninfected TB contacts with two TST or QFTGIT measurements.

3.3. Vitamin D Determinations

Levels of 25-hydroxyvitamin D (25 [OH] D) in serum were measured by two techniques: electrochemiluminescence (ECLIA) on a COBAS® 410 Roche analyzer from 2009 to 2010 (202 contacts), and chemiluminescence immunoassay (CLIA) on a IDS-iSYS automated analyzer from 2011 to 2012 (370 contacts) in the Biochemical laboratory of the HGC (21, 22). An extra 10% of concentration of VitD was added for the patients studied by ECLIA, because the ECLIA technique measures only D3 and not D2. Supplements of VitD were recommended for contacts with insufficient VitD levels (< 30 ng/mL).

3.4. Ethical Issues

The Ethics Committee of the HGC approved the study, and signed consent was obtained from all participants or their parents. Children under the age of 10 years were not included in the study.

3.5. Statistical Analysis

The five groups were compared using the Chi2 and Fisher tests for qualitative variables and the Kruskal-Wallis test for quantitative variables. Multinomial logistic regression (MLR) models were used in order to quantify the relative risk (RR) of the five groups (dependent variables) in accordance with different independent variables, taking the

group uninfected with TB and with two TST or QFTGIT measurements as the reference group. Two MLRs were implemented, with the first considering the two first groups and the second including the two remaining groups, using the last group as a reference in all cases. All MLR models meet the property of independence from irrelevant alternatives following the generalized Hausman specification test (23). The Stata® program version 12.0 was used for the statistical analysis.

4. Results

During the study period, 836 contacts of pulmonary TB patients were screened, and 572 who had serum VitD determinations were included in the study. Among them, 523 (91.4%) completed the follow-up and were divided into five contact groups (Table1): 1) 3 (0.6%) new pulmonary TB patients with a positive M. tuberculosis sputum culture; 2) 27 (5.2%) TBIC patients; 3) 125 (23.9%) LTBI patients; 4) 116 (22.2%) uninfected TB contacts when only a TST or a QFTGIT was required (41 contacts) or contacts without a second TST or QFTGIT determination (75 contacts); and 5) 252 (48.2%) uninfected TB contacts with two TST or QFTGIT measurements.

Table 1 shows the characteristics of the groups. Values for age, residence in Vila-real, and social class III-VI were higher in the new pulmonary TB patients; the foreign-born group had higher proportions of TBIC patients. The mean of VitD was lower for the new pulmonary TB patients, and the higher value corresponded to the group of uninfected TB contacts with two TST or QFTGIT measurements. This last group had the highest proportion (38.1%) of contacts with sufficient VitD levels ($\geq$ 30 ng/mL); only 14.8% of the TBIC patients and 0% of the new pulmonary TB patients had sufficient levels. Among all participants, the prevalence of sufficient, deficient (< 20 ng/mL), and very deficient (< 10 ng/mL) VitD levels was 34.0%, 24.9% and 7.8%, respectively. The new pulmonary TB patients had the highest exposure and a sputum AFB-positive index case. Other variables, including BCG vaccination, BMI, diabetes mellitus, current smoker, and study period were not associated with the groups. Chemoprophylaxis was recommended for 84 contacts (14.7%).

The MLR (Table 2) included all groups, with the uninfected TB contacts with two TSTs or QFTGITs group as the reference. VitD status and high exposure and a sputum AFB-positive index case were significantly associated with three or more groups, while VitD status was a significant protector against new pulmonary TB (RR = 0.86; 95% CI 0.74 - 0.99) and TBIC (RR = 0.95; 95% CI 0.91 - 0.99). Pulmonary TB and TBIC risks were reduced by 14% and 5% by an increase of 1 ng/mL of VitD, respectively. The combination of high exposure and a sputum AFB-positive index case was associated with pulmonary TB disease, TBIC, and LTBI. However, VitD status was not associated with LTBI (RR = 0.99; 95% CI 0.97-1.01).

An MLR model considering only the three groups of pulmonary TB, TBIC, and uninfected TB contacts with two TSTs or QFTGITs was carried out (Table 3); VitD status was a protective factor against pulmonary TB and TBIC. Male gender and being foreign-born were risk factors for TBIC (RR = 3.04; 95% CI 1.22 - 7.59 and RR = 2.62; 95% CI 1.07 - 6.40, respectively). The combination of high exposure and a sputum AFB-positive index case was a high risk factor.

Significant differences were found when the LTBI patients and uninfected TB with one TST or QFTGIT measurement groups were compared with the reference group (Table 4). The LTBI patients group was older, included more current smokers, and had an increase in high exposure and sputum AFB-positive index cases; Vila-real residence was a protective factor. VitD status was not associated with LTBI. In the uninfected TB contacts with one TST or QFTGIT measurement group, VitD status and Vila-real residence were a protective factor.

5. Discussion

In this study, we have found VitD status as a protective factor against pulmonary TB and TBIC, but not for LTBI. Considering all variables, the variables associated with TB in three groups were a high exposure sputum AFB-positive index case and VitD status. In this cohort, the incidence of pulmonary TB was higher than other studies, but the frequency of TBIC and LTBI were lower; on the other hand, the number of cases lost to follow-up was low (24-26).

Variables such as age, male gender, foreign birthplace, social class, malnutrition, being a current smoker, and season have been associated with TB in different studies (27-31). However, in the MLR models, these factors lost significance, suggesting that VitD status is strongly associated with TB outcomes. In more restricted comparisons, male gender and foreign birthplace were associated with TBIC, whereas age and being a current smoker were associated with LTBI.

Few studies have explored VitD status before the development of TBIC or TB disease. These include a clinical trial in Mongolian children on TBIC (14), a study of TB disease in Pakistan (4), and another study in Africa with HIV patients (15). In these studies, low VitD levels were associated with an increase of TB. Studies on active TB patients suggested that low VitD status is a risk factor for the disease (32-34).

We have found that the prevalence of LTBI was not associated with VitD status. Serum VitD status can vary with

Table 1. Characteristic Distribution and Comparison of the Five Groups of Study[a]

Variables	New Pulmonary TB Patients	TB Infection Conversion Patients	Latent TB Infection Patients	Uninfected TB Contacts With one TST or QFTGIT Measurement	Uninfected TB Contacts With Two TST or QFTGIT Measurements	P-Value
	N = 3	N = 27	N = 125	N = 116	N = 252	
Age, Mean ± SD	42.3 ± 24.0	32.1 ± 15.4	41.2 ± 15.7	37.2 ± 12.3	37.2 ± 13.3	0.026
Male	2 (66.7)	19 (67.9)	82 (59.4)	78 (56.1)	129 (48.9)	0.173
Vila-real residence	2 (66.7)	9 (33.3)	18 (14.4)	10 (8.6)	57 (22.6)	0.000
Foreign-born	1 (33.3)	12 (44.4)	40 (32.0)	40 (34.5)	57 (22.6)	0.034
Social class III-VI	3 (100.0)	24 (88.9)	114 (91.2)	92 (79.3)	199 (79.0)	0.022
BCG vaccination	1 (33.3)	14 (51.9)	59 (47.2)	51 (44.0)	100 (39.7)	0.549
BMI, Mean ±SD	26.4 ± 3.1	23.8 ± 4.4	26.1 ± 4.7	24.6 ± 3.9	25.6 ± 5.1	0.174
Diabetes mellitus	1 (33.3)	0 (0)	4 (3.2)	4 (3.4)	10 (4.0)	0.250
Current smoker[b]	1 (33.3)	8 (29.6)	49 (43.0)	33 (30.6)	79 (31.3)	0.267
Study period Apr.-Sept. versus May-Oct.	2 (66.7)	9 (33.3)	60 (48.0)	60 (51.7.9)	133 (52.8)	0.354
25 (OH) vitamin D ng/mL, Mean ± SD	14.2 ± 6.7	20.9 ± 10.0	26.4 ± 13.8	25.1 ± 14.5	27.8 ± 12.4	0.003
25 (OH) vitamin D levels, ng/ml						
0 - 9	1 (33.3)	3 (11.1)	14 (11.2)	12 (10.3)	12 (4.8)	
10 - 19	2 (66.7)	11 (40.7)	28 (22.4)	32 (27.6)	57 (22.6)	
20 - 29	0 (0)	9 (33.3)	38 (30.4)	39 (33.6)	87 (34.5)	
≥ 30	0 (0)	4 (14.8)	45 (36.0)	33 (28.4)	96 (38.1)	0.002
Exposure to TB index case hour/day						
High > 6	3 (100.0)	13(48.1)	43 (34.4)	16 (13.8)	32 (12.7)	
Medium 1 - 6	0 (0)	10 (37.0)	43 (34.4)	23 (19.8)	110 (43.7)	
Low < 1	0 (0)	4 (14.8)	39 (31.2)	77 (66.4)	110 (43.7)	0.001
Sputum AFB-positive index case (%)	2 (66.7)	20 (74.1)	98 (78.4)	82 (70.7)	202 (80.2)	0.343
High exposure and sputum AFB-positive index case	2 (66.7)	8 (29.6)	31(24.8)	8(6.9)	18 (7.1)	0.000
Chemoprophylaxis against TB	0 (0)	21 (77.7)	61 (48.8)	0 (0)	2 (0.08)	0.000

Abbreviations: BCG, bacilli Calmette-Guerin; BMI, body mass index; TB, tuberculosis; TST, tuberculin skin test; SD, standard deviation; social class III-VI, skilled non-manual and manual, partially skilled and unskilled; QFTGIT, QuantiFERON-TB Gold in-tube test.

[a] Values are expressed as No. (%) unless otherwise indicated.

[b] Some groups without complete information.

the seasons of the year, and the onset of LTBI may be prior to the studied TB exposure. In addition, LTBI may require sufficient VitD levels to have antimicrobial activity against M. tuberculosis in order to prevent active TB (35), and other unknown factors apart from VitD could play a role in TB development (36).

VitD plays an important role in innate and adaptive immunity protection against TB. Multiple cells, receptors, cytokines, and antimicrobial peptides such as cathelicidin and defensin beta 4 have been implicated in these complex mechanisms (37-41). In this context, a sufficient VitD level at the time of *M. tuberculosis* exposure could be crucial to obtain an adequate protection against TBIC, together with an optimal nutritional status (42).

It should be noted that the prevalence of very deficient and deficient VitD status (< 30 ng/mL) was high, considering the localization of Castellon and the Mediterranean climate. This is in line with some studies performed in Spain, which have found a high prevalence of deficient levels of VitD in several populations (43-45).

Turning to the strengths of this study, we highlight its prospective design, an acceptable participation and follow-up rate, contacts with exposure to pulmonary TB, validated techniques of VitD measurement, and the two methods to test the TB situation, namely the TST and the QFTGIT.

In terms of the study limitations, these include the lack of VitD receptor polymorphisms genotyping and the use of only one determination of VitD with two different techniques. Among the TB risk factors, measurements that are more precise, such as poverty or crowding, could have been used. In addition, the sample of new pulmonary TB patients was small.

On the other hand, the measurement of TBIC has some

Table 2. Multinomial Logistic Regression by All Groups of Study[a]

	Variables	RR	95% CI	P-Value
New TB Disease Patients				
N = 3	Vitamin D ng/mL	0.86	0.74 - 0.99	0.041
	High exposure and sputum AFB-positive index case	36.72	2.90 - 439.98	0.005
TB infection conversion patients				
N = 27	Vitamin D ng/mL	0.95	0.91 - 0.99	0.008
	High exposure and sputum AFB-positive index case	5.94	2.26 - 15.77	0.000
Latent TB infection patients				
N = 116	Vitamin D ng/mL	0.99	0.97 - 1.01	0.350
	High exposure and sputum AFB-positive index case	4.29	2.29 - 8.04	0.000
Uninfected TB contacts with one TST or QFTGIT measurement				
N = 125	Vitamin D ng/mL	0.98	0.96 - 1.00	0.078
	High exposure and sputum AFB-positive index case	0.97	0.41 - 2.30	0.944
Uninfected TB contacts with two TST or QFTGIT measurements	Reference group			
N = 252				

Abbreviations: AFB, acid-fast bacilli; CI, confidence interval; RR, relative risk; TB, tuberculosis; TST, tuberculin skin test; QFTGIT, QuantiFERON-TB Gold in-tube test.
[a]Uninfected TB contacts after two TST or QFTGIT measurements are the reference group.

Table 3. Multinomial Logistic Regression: New Pulmonary TB Patients, TB Infection Conversion Patients, and Uninfected TB Contacts With Two TST or QFTGIT Measurements as a Reference Group

	Variables	RR	95% CI	P-Value
New pulmonary TB patients				
N = 3	Vitamin D ng/mL	0.81	0.66 - 0.99	0.039
	High exposure and sputum AFB-positive index case	63.34	3.90 - 1029.59	0.003
	Male	2.45	0.17 - 34.35	0.507
	Foreign-born	0.68	0.03 - 14.59	0.807
TB infection conversion patients				
N = 27	Vitamin D ng/mL	0.93	0.89 - 0.98	0.003
	High exposure and sputum AFB-positive index case	7.12	2.39 - 21.20	0.000
	Male	3.04	1.22 - 7.59	0.017
	Foreign-born	2.62	1.07 - 6.40	0.034
Uninfected TB contacts with two TST or QFTGIT measurements	Reference group			
N = 252				

Abbreviations: CI, confidence interval; RR, relative risk; TB, tuberculosis; TST, tuberculin skin test; QFTGIT, QuantiFERON-TB Gold in-tube test.

complexity for the TST and the QFTGIT (46, 47), and the VitD status determination presents technical problems in relation to the carrier, the vitamin D binding protein, and the standardization (48). One direction for future research into the effect of VitD on TB incidence (TBIC or disease) would be to introduce a new measurement of VitD at the time of the second screening for TB control.

Determining VitD status among the contacts of TB patients could be useful to evaluate the risk of TB development, and VitD supplements could be recommended if a VitD deficiency is found, considering the high prevalence of deficient levels and their relationship to TB. Two preventive clinical trials have been proposed to establish whether supplements of VitD could prevent TB infections (49).

Table 4. Multinomial Logistic Regression: Latent TB Infection Patients, Uninfected TB Contacts With One TST or QFTGIT Measurement, and Uninfected TB Contacts With Two TST or QFTGIT Measurements as a Reference Group

	Variables	RR	95% CI	P-value
Latent TB infection patients				
N = 125	Vitamin D ng/mL	0.99	0.98 - 1.02	0.798
	High exposure and sputum AFB-positive index case	4.55	2.32 - 8.90	0.000
	Age (years)	1.03	1.01 - 1.05	0.001
	Vila-real residence	0.55	0.31 - 1.11	0.058
	Current smoker	1.88	1.15 - 3.07	0.012
Uninfected TB contacts with one TST or QFTGIT measurement				
N = 116	Vitamin D ng/mL	0.98	0.96 - 0.99	0.019
	High exposure and sputum AFB-positive index case	1.04	0.43 - 2.52	0.926
	Age (years)	1.00	0.98 - 1.02	0.795
	Vila-real residence	0.32	0.16 - 0.66	0.002
	Current smoker	0.88	0.53 - 1.45	0.610
Uninfected TB contacts with two TST or QFTGIT measurements	Reference group			
N = 252				

Abbreviations: CI, confidence interval; RR, relative risk; TB, tuberculosis; TST, tuberculin skin test; QFTGIT, QuantiFERON-TB Gold in-tube test.

By way of conclusion, the results suggest that a sufficient VitD level could be a protective factor against TBIC and pulmonary TB.

Acknowledgments

The authors thank the participants for their generous cooperation in making this study possible. The authors are grateful to Rita Holguin-Gomez and Esther Silvestre-Silvestre for their contribution to this study.

Footnotes

Authors' Contribution: Study design and statistical analysis were supplied by Alberto Arnedo-Pena (AAP) and Maria Angeles Romeu-Garcia (MARG); MARG, Juan B. Bellido-Blasco (JBBB), and Noemi Meseguer-Ferrer carried out data collection; Jose Vicente Juan-Cerdan (JVJC), Daniel Garcia-Ferrer (DGF), Jesus Iborra-Millet (JIM), Jose Antonio Ferrero-Vega (JAFV), and Francisco Pardo-Serrano (FPS) performed the laboratory tests; AAP, MARG, and JBBB contributed to writing up the manuscript; and the revision of the manuscript was handled by JVJC, DGF, JIM, JAFV, and FPS.

Financial Disclosure: Authors declare no conflicts of interest.

References

1. Davies PD, Martineau AR. Vitamin D and tuberculosis: more effective in prevention than treatment?. *Int J Tuberc Lung Dis.* 2015;**19**(8):876–7. doi: 10.5588/ijtld.15.0506. [PubMed: 26162349].
2. Nnoaham KE, Clarke A. Low serum vitamin D levels and tuberculosis: a systematic review and meta-analysis. *Int J Epidemiol.* 2008;**37**(1):113–9. doi: 10.1093/ije/dym247. [PubMed: 18245055].
3. Zeng J, Wu G, Yang W, Gu X, Liang W, Yao Y, et al. A serum vitamin D level <25nmol/l pose high tuberculosis risk: a meta-analysis. *PLoS One.* 2015;**10**(5):0126014. doi: 10.1371/journal.pone.0126014. [PubMed: 25938683].
4. Talat N, Perry S, Parsonnet J, Dawood G, Hussain R. Vitamin d deficiency and tuberculosis progression. *Emerg Infect Dis.* 2010;**16**(5):853–5. doi: 10.3201/eid1605.091693. [PubMed: 20409383].
5. Sudfeld CR, Giovannucci EL, Isanaka S, Aboud S, Mugusi FM, Wang M, et al. Vitamin D status and incidence of pulmonary tuberculosis, opportunistic infections, and wasting among HIV-infected Tanzanian adults initiating antiretroviral therapy. *J Infect Dis.* 2013;**207**(3):378–85. doi: 10.1093/infdis/jis693. [PubMed: 23162137].
6. Tukvadze N, Sanikidze E, Kipiani M, Hebbar G, Easley KA, Shenvi N, et al. High-dose vitamin D3 in adults with pulmonary tuberculosis: a double-blind randomized controlled trial. *Am J Clin Nutr.* 2015;**102**(5):1059–69. doi: 10.3945/ajcn.115.113886. [PubMed: 26399865].
7. Daley P, Jagannathan V, John KR, Sarojini J, Latha A, Vieth R, et al. Adjunctive vitamin D for treatment of active tuberculosis in India: a randomised, double-blind, placebo-controlled trial. *Lancet Infect Dis.* 2015;**15**(5):528–34. doi: 10.1016/S1473-3099(15)70053-8. [PubMed: 25863562].
8. Martineau AR, Timms PM, Bothamley GH, Hanifa Y, Islam K, Claxton AP, 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. [PubMed: 21215445].

9. Wejse C, Gomes VF, Rabna P, Gustafson P, Aaby P, Lisse IM, et al. Vitamin D as supplementary treatment for tuberculosis: a double-blind, randomized, placebo-controlled trial. *Am J Respir Crit Care Med.* 2009;**179**(9):843–50. doi: 10.1164/rccm.200804-567OC. [PubMed: 19179490].
10. Ralph AP, Waramori G, Pontororing GJ, Kenangalem E, Wiguna A, Tjitra E, et al. L-arginine and vitamin D adjunctive therapies in pulmonary tuberculosis: a randomised, double-blind, placebo-controlled trial. *PLoS One.* 2013;**8**(8):70032. doi: 10.1371/journal.pone.0070032. [PubMed: 23967066].
11. Salahuddin N, Ali F, Hasan Z, Rao N, Aqeel M, Mahmood F. Vitamin D accelerates clinical recovery from tuberculosis: results of the SUCCINCT Study [Supplementary Cholecalciferol in recovery from tuberculosis]. A randomized, placebo-controlled, clinical trial of vitamin D supplementation in patients with pulmonary tuberculosis'. *BMC Infect Dis.* 2013;**13**:22. doi: 10.1186/1471-2334-13-22. [PubMed: 23331510].
12. Mily A, Rekha RS, Kamal SM, Arifuzzaman AS, Rahim Z, Khan L, et al. Significant Effects of Oral Phenylbutyrate and Vitamin D3 Adjunctive Therapy in Pulmonary Tuberculosis: A Randomized Controlled Trial. *PLoS One.* 2015;**10**(9):0138340. doi: 10.1371/journal.pone.0138340. [PubMed: 26394045].
13. Xia J, Shi L, Zhao L, Xu F. Impact of vitamin D supplementation on the outcome of tuberculosis treatment: a systematic review and meta-analysis of randomized controlled trials. *Chin Med J (Engl).* 2014;**127**(17):3127–34. [PubMed: 25189958].
14. Ganmaa D, Giovannucci E, Bloom BR, Fawzi W, Burr W, Batbaatar D, et al. Vitamin D, tuberculin skin test conversion, and latent tuberculosis in Mongolian school-age children: a randomized, double-blind, placebo-controlled feasibility trial. *Am J Clin Nutr.* 2012;**96**(2):391–6. doi: 10.3945/ajcn.112.034967. [PubMed: 22760564].
15. Arnedo-Pena A, Juan-Cerdan JV, Romeu-Garcia A, Garcia-Ferrer D, Holguin-Gomez R, Iborra-Millet J, et al. Latent tuberculosis infection, tuberculin skin test and vitamin D status in contacts of tuberculosis patients: a cross-sectional and case-control study. *BMC Infect Dis.* 2011;**11**:349. doi: 10.1186/1471-2334-11-349. [PubMed: 22171844].
16. Arnedo-Pena A, Juan-Cerdan JV, Romeu-Garcia MA, Garcia-Ferrer D, Holguin-Gomez R, Iborra-Millet J, et al. Vitamin D status and incidence of tuberculosis infection conversion in contacts of pulmonary tuberculosis patients: a prospective cohort study. *Epidemiol Infect.* 2015;**143**(8):1731–41. doi: 10.1017/S0950268814002386. [PubMed: 25274036].
17. Arnedo-Pena A, Juan-Cerdan JV, Romeu-Garcia A, Garcia-Ferrer D, Holguin-Gomez R, Iborra-Millet J, et al. Vitamin D status and incidence of tuberculosis among contacts of pulmonary tuberculosis patients. *Int J Tuberc Lung Dis.* 2015;**19**(1):65–9. doi: 10.5588/ijtld.14.0348. [PubMed: 25519792].
18. National consensus on the control of tuberculosis in Spain. Task Group on Tuberculosis [in Spanish]. *Med Clin (Barc).* 1992;**98**(1):24–31. [PubMed: 1545607].
19. Tuberculosis Technical Commission . Program for prevention and control of tuberculosis. Valencia: Consum Health Department Valencia; 1993.
20. Mellado Peña M. Interpretación de la prueba de tuberculina en niños. *Anales de Pediatría.* 2003;**59**(6):582–5. doi: 10.1016/s1695-4033(03)78783-9.
21. Leino A, Turpeinen U, Koskinen P. Automated measurement of 25-OH vitamin D3 on the Roche Modular E170 analyzer. *Clin Chem.* 2008;**54**(12):2059–62. doi: 10.1373/clinchem.2008.111732. [PubMed: 18927245].
22. Cluse ZN, Fudge AN, Whiting MJ, McWhinney B, Parkinson I, O'Loughlin PD. Evaluation of 25-hydroxy vitamin D assay on the immunodiagnostic systems iSYS analyser. *Ann Clin Biochem.* 2012;**49**(Pt 2):159–65. doi: 10.1258/acb.2011.011018. [PubMed: 22155920].
23. Hausman J, McFadden D. Specification Tests for the Multinomial Logit Model. *Econometrica.* 1984;**52**(5):1219. doi: 10.2307/1910997.
24. Fox GJ, Barry SE, Britton WJ, Marks GB. Contact investigation for tuberculosis: a systematic review and meta-analysis. *Eur Respir J.* 2013;**41**(1):140–56. doi: 10.1183/09031936.00070812. [PubMed: 22936710].
25. Cailleaux-Cezar M, de AD, Xavier GM, de Salles CL, de Mello FC, Ruffino-Netto A, et al. Tuberculosis incidence among contacts of active pulmonary tuberculosis. *Int J Tuberc Lung Dis.* 2009;**13**(2):190–5. [PubMed: 19146746].
26. Gorís-Pereiras A, Fernández-Villar A, Chouciño-Garrido N, Otero-Baamonde M, Vázquez-Gallardo R. Factores predictores de la aparición de nuevos casos de infección tuberculosa y de viraje tuberculínico en un estudio de contactos. *Enfermería Clínica.* 2008;**18**(4):183–9. doi: 10.1016/s1130-8621(08)72193-4.
27. Pinheiro RS, de Oliveira GP, Oliveira EX, Melo EC, Coeli CM, Carvalho MS. Social determinants and self-reported tuberculosis: National Research by Household Sample, metropolitan areas, Brazil [in Portuguese]. *Rev Panam Salud Publica.* 2013;**34**(6):446–51. [PubMed: 24569974].
28. Moran-Mendoza O, Marion SA, Elwood K, Patrick D, FitzGerald JM. Risk factors for developing tuberculosis: a 12-year follow-up of contacts of tuberculosis cases. *Int J Tuberc Lung Dis.* 2010;**14**(9):1112–9. [PubMed: 20819255].
29. Feng JY, Huang SF, Ting WY, Lee MC, Chen YC, Lin YY, et al. Impact of cigarette smoking on latent tuberculosis infection: does age matter?. *Eur Respir J.* 2014;**43**(2):630–2. doi: 10.1183/09031936.00118313. [PubMed: 24072215].
30. Neyrolles O, Quintana-Murci L. Sexual inequality in tuberculosis. *PLoS Med.* 2009;**6**(12):1000199. doi: 10.1371/journal.pmed.1000199. [PubMed: 20027210].
31. Wingfield T, Schumacher SG, Sandhu G, Tovar MA, Zevallos K, Baldwin MR, et al. The seasonality of tuberculosis, sunlight, vitamin D, and household crowding. *J Infect Dis.* 2014;**210**(5):774–83. doi: 10.1093/infdis/jiu121. [PubMed: 24596279].
32. Esteve Palau E, Sanchez Martinez F, Knobel Freud H, Lopez Colomes JL, Diez Perez A. Tuberculosis: plasma levels of vitamin D and its relation with infection and disease [in Spanish]. *Med Clin (Barc).* 2015;**144**(3):111–4. doi: 10.1016/j.medcli.2013.09.036. [PubMed: 24361157].
33. Junaid K, Rehman A, Saeed T, Jolliffe DA, Wood K, Martineau AR. Genotype-independent association between profound vitamin D deficiency and delayed sputum smear conversion in pulmonary tuberculosis. *BMC Infect Dis.* 2015;**15**:275. doi: 10.1186/s12879-015-1018-5. [PubMed: 26193879].
34. Sloan DJ, Mwandumba HC, Kamdolozi M, Shani D, Chisale B, Dutton J, et al. Vitamin D deficiency in Malawian adults with pulmonary tuberculosis: risk factors and treatment outcomes. *Int J Tuberc Lung Dis.* 2015;**19**(8):904–11. doi: 10.5588/ijtld.15.0071. [PubMed: 26162355].
35. Montoya D, Inkeles MS, Liu PT, Realegeno S, Teles RM, Vaidya P, et al. IL-32 is a molecular marker of a host defense network in human tuberculosis. *Sci Transl Med.* 2014;**6**(250):114–250. doi: 10.1126/scitranslmed.3009546. [PubMed: 25143364].
36. Offer C, Lee A, Humphreys C. Tuberculosis in South Asian communities in the UK: a systematic review of the literature. *J Public Health (Oxf).* 2016;**38**(2):250–7. doi: 10.1093/pubmed/fdv034. [PubMed: 25818340].
37. Liu PT, Stenger S, Li H, Wenzel L, Tan BH, Krutzik SR, et al. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. *Science.* 2006;**311**(5768):1770–3. doi: 10.1126/science.1123933. [PubMed: 16497887].
38. Guo C, Gombart AF. The antibiotic effects of vitamin D. *Endocr Metab Immune Disord Drug Targets.* 2014;**14**(4):255–66. [PubMed: 25008764].
39. Schwander S, Ellner J. Human response to M.Tuberculosis. In: Davies PDO, Barnes PP, Gordon SB, editors. Clinical tuberculosis. London: Hodder Education; 2008. .
40. Hewison M. An update on vitamin D and human immunity. *Clin Endocrinol (Oxf).* 2012;**76**(3):315–25. doi: 10.1111/j.1365-2265.2011.04261.x.

[PubMed: 21995874].
41. Weiss G, Schaible UE. Macrophage defense mechanisms against intracellular bacteria. *Immunol Rev.* 2015;**264**(1):182–203. doi: 10.1111/imr.12266. [PubMed: 25703560].
42. Cegielski P, Vernon A. Tuberculosis and vitamin D: what's the rest of the story?. *Lancet Infect Dis.* 2015;**15**(5):489–90. doi: 10.1016/S1473-3099(15)70163-5. [PubMed: 25863560].
43. Gonzalez-Molero I, Morcillo S, Valdes S, Perez-Valero V, Botas P, Delgado E, et al. Vitamin D deficiency in Spain: a population-based cohort study. *Eur J Clin Nutr.* 2011;**65**(3):321–8. doi: 10.1038/ejcn.2010.265. [PubMed: 21179052].
44. Gonzalez-Padilla E, Soria Lopez A, Gonzalez-Rodriguez E, Garcia-Santana S, Mirallave-Pescador A, Groba Marco Mdel V, et al. [High prevalence of hypovitaminosis D in medical students in Gran Canaria, Canary Islands (Spain)]. *Endocrinol Nutr.* 2011;**58**(6):267–73. doi: 10.1016/j.endonu.2011.03.002. [PubMed: 21555257].
45. Almirall J, Vaqueiro M, Bare ML, Anton E. Association of low serum 25-hydroxyvitamin D levels and high arterial blood pressure in the elderly. *Nephrol Dial Transplant.* 2010;**25**(2):503–9. doi: 10.1093/ndt/gfp470. [PubMed: 19749143].
46. Look VJ, Fitzgerald JM, Menzies D. Clinical interpretation of tests for latent tuberculosis infection. In: Davies PDO, Barnes PP, Gordon SB, editors. Clinical tuberculosis. 4 ed. London: Hodder Education; 2008. pp. 393–410.
47. Zanetti C, Peracchi M, Zorzi D, Fiorio S, Fallico L, Palu G. Outbreak of transient conversions of the QuantiFERON-TB Gold In-Tube test in laboratory health care worker screenings. *Clin Vaccine Immunol.* 2012;**19**(6):954–60. doi: 10.1128/CVI.05718-11. [PubMed: 22518010].
48. Romagnoli E, Pepe J, Piemonte S, Cipriani C, Minisola S. Management of endocrine disease: value and limitations of assessing vitamin D nutritional status and advised levels of vitamin D supplementation. *Eur J Endocrinol.* 2013;**169**(4):59–69. doi: 10.1530/EJE-13-0435. [PubMed: 23847326].
49. Turnbull ER, Drobniewski F. Vitamin D supplementation: a comprehensive review on supplementation for tuberculosis prophylaxis. *Expert Rev Respir Med.* 2015;**9**(3):269–75. doi: 10.1586/17476348.2015.1042458. [PubMed: 25959993].