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Original Article

Revisiting the association between vitamin D deficiency and active tuberculosis: A prospective case-control study in Taiwan

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Abstract *Background:* To revisit the association between vitamin D deficiency (VDD, defined as serum 25(OH)D $<$ 20 ng/ml) and incident active tuberculosis (TB), after two potentially underpowered randomized trials showed statistically non-significant 13%–22% decrease in TB incidence in vitamin D supplementation groups.
Methods: We prospectively conducted an age/sex-matched case–control study that accounting for body-mass index (BMI), smoking, and other confounding factors to examine the association between VDD and active TB among non-HIV people in Taiwan (latitude 24°N), a high-income society which continues to have moderate TB burden.

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Results: We enrolled 62 people with incident active TB and 248 people in control group. The TB case patients had a significantly higher proportion of VDD compared to the control group (51.6% vs 29.8%, $p = 0.001$). The 25(OH)D level was also significantly lower in TB patients compared to control group (21.25 ± 8.93 ng/ml vs 24.45 ± 8.36 ng/ml, $p = 0.008$). In multivariable analysis, VDD (adjusted odds ratio [aOR]: 3.03, $p = 0.002$), lower BMI (aOR: 0.81, $p < 0.001$), liver cirrhosis (aOR: 8.99, $p = 0.042$), and smoking (aOR: 4.52, $p = 0.001$) were independent risk factors for incident active TB.
Conclusions: VDD is an independent risk factor for incident active TB. Future randomized trials examining the effect of vitamin D supplementation on TB incidence should focus on people with a low BMI or other risk factors to maximize the statistical power.

Introduction

Even during the coronavirus disease 2019 (COVID-19) pandemic, tuberculosis (TB) remains a leading cause of communicable disease-associated deaths worldwide.[1] The World Health Organization estimated that 10.6 million people fell ill with TB in 2021, an increase of 4.5% from 10.1 million in 2020.[1] The rising of incidence rate of TB between 2020 and 2021, probably caused by disruption of TB preventive and treatment service by the COVID-19 pandemic, sets back the aim of the Global End TB strategy. There is an unmet need to reduce global TB burden.

Vitamin D is a key modulator for both innate and adaptive immunity against *Mycobacterium tuberculosis*.[2] *M. tuberculosis* is highly infectious, but there are substantial variations in individual's susceptibility, probably involving complex interactions between environmental, genetic, socioeconomic, and nutritional factors.[3] Studies on genetic susceptibility to TB have focused on polymorphisms of different candidate genes, with a particular interest in vitamin D receptor.[4] These findings support the role of vitamin D in mitigating the pathogenesis of TB.

However, the association between vitamin D deficiency (VDD, defined as serum 25(OH)D concentration <20 ng/ml) and TB remains controversial. Previous studies yielded conflicting results. Studies from populations in Vietnam,[5] Malawi,[6] and South Korea[7] showed no significant association between VDD and TB. On the other hand, reports from Chile[8] and India[9] showed a significant association between lower vitamin D levels and susceptibility to TB. Meta-analyses reveal high heterogeneity between previous studies, likely because of uncontrolled confounders.[10—12] Two potentially underpowered randomized controlled trials that examined the effect of vitamin D supplementation in Mongolian children and Tanzanian people living with HIV had shown a statistically non-significant 13%—22% decrease in incidence of active TB in vitamin D supplementation group, respectively.[13,14]

We aimed to revisit the association between VDD and incident active TB, and to identify important confounding factors that need to be considered in designing future randomized trials. To maximize the statistical power, we conducted an individually age/sex-matched prospective case—control study in Taiwan, a high-income society which continues to have moderate TB burden (annual TB incidence 30.1 per 100,000 population in 2021).

Methods

Study setting and design

The population of this study comprised ambulatory people living in northern Taiwan (latitude 24°N). We enrolled all cases and controls at Far Eastern Memorial Hospital, which is a tertiary medical center in northern Taiwan. The hospital has approximately 100 new TB diagnoses annually during the period from 2018 to 2021. Vitamin D concentration has not yet become a routine test for individuals diagnosed with TB. This study was approved by the Research Ethics Review Committee of Far Eastern Memorial Hospital (IRB number 110133-E), and written informed consent was obtained from all participants.

Eligibility criteria

From September 15, 2021 to December 14, 2022, people with a new TB diagnosis who were receiving anti-TB treatment during the enrollment period were eligible to be enrolled as cases if they were aged over 20 and had microbiological evidence of TB. After enrolling the cases of tuberculosis (TB), serum 25(OH)D levels were tested within one month. For each case, four age- and sex-matched patients without active TB disease or a history of active TB, and not taking vitamin D or any other kinds of fat-soluble vitamin supplements within one month, were enrolled as controls. Controls were enrolled from cardiovascular clinics, health check-up clients in the Preventive Health Center, and healthcare workers. Pregnant women and people living with HIV and were not eligible. Patients with notified latent TB were excluded. To control the effect of sunlight exposure levels, those who were not residents in northern Taiwan were excluded. Once the controls were enrolled, serum 25(OH)D levels were tested within three months.

Data collection

The demographic data and BMI were collected by using a computerized electronic format. All participants underwent a detailed medical history collection, including underlying diseases, alcohol consumption, and cigarette smoking history. To define smoking status, we grouped

patients into either smoker (i.e., smoked cigarettes at the time of enrollment or had previously smoked more than 100 cigarettes in their lifetime) or non-smoker (i.e., never smoked or had smoked <100 cigarettes in their lifetime).[15] The clinical data, including TB sites, anti-TB therapy, grading of acid-fast bacilli (AFB) stain,[16] time to AFB and culture negative conversion, and chest X-ray (CXR) or chest computer tomography findings on the day of diagnosis, were collected in people with TB.

Vitamin D concentration and status definition

The serum 25(OH)D level was measured using the Cobas® e 801 Module (Roche Diagnostics) according to the manufacturer's protocols. The level of 25(OH)D was expressed in nanograms per milliliter (ng/ml). We graded the 25(OH)D level into four categories according to the Endocrine Society Clinical Practice Guidelines.[17] Briefly, VDD was defined as the 25(OH)D level <20 ng/ml. Further, "severe deficiency" and "moderate deficiency" were defined as the 25(OH)D level <10 ng/ml and the level between 10 ng/ml to 20 ng/ml, respectively. For the 25(OH)D level of 20–30 ng/ml and level ≥30 ng/ml, the vitamin D status was defined as "insufficiency" and "sufficiency", respectively.

Statistical analysis

Statistical analyses were performed using SAS 9.4 (Cary, North Carolina, USA). Categorical variables were expressed as percentages, and continuous variables were expressed as means ± standard deviations. Chi-squared test was used to compare two proportions. Fisher's exact test was used when any value in the cells of the contingency table was smaller than five. Continuous data were compared using Student's t-test. Conditional logistic regression was used to analyze the matched data. For multivariable analysis, all variables with a p-value <0.10 in the univariable analysis were included in the maximum model, and stepwise selection was used to decide the optimum model. All statistical tests were two-tailed, and $p < 0.05$ was considered statistically significant.

Results

TB patients

We enrolled 62 patients with incident active TB (cases), including 21 diagnosed before the start of enrollment but were still receiving anti-TB therapy during the enrollment period and 41 diagnosed during the enrollment period. All of the 21 individuals diagnosed with TB before enrollment and still receiving anti-TB medications had first-time active TB disease. Fig. 1A shows the enrollment process. The 62 patients had an average age of 58.5 ± 14.3 years old, and 44 (71%) of them were male (Table 1). Nearly all of the enrolled people had pulmonary TB (59/62, 95%), and only 3 (5%) patients had extrapulmonary TB, including one with TB meningitis and the other two with TB lymphadenitis of neck. The average 25(OH)D concentration of the 62 patients was 21.25 ± 8.93 ng/ml, and among them, 51.6% (32/62) patients had VDD. Other baseline demographic characteristics and underlying medical diseases were shown in Table 1.

Controls

We enrolled a total of 248 subjects without active TB (individually matched to cases in 1:4 ratio by age and sex) as the controls, including 154 cases (60%) from cardiovascular clinics, 35 cases (13.6%) from Preventive Health Center, and 68 cases (26.5%) from health-care workers (Fig. 1B). The control group included 176 men and 72 women, with an average age of 58.6 ± 14.5 years. Overall, VDD was present in 34.2% (106/310) of all participants. Other characteristics were shown in Table 1.

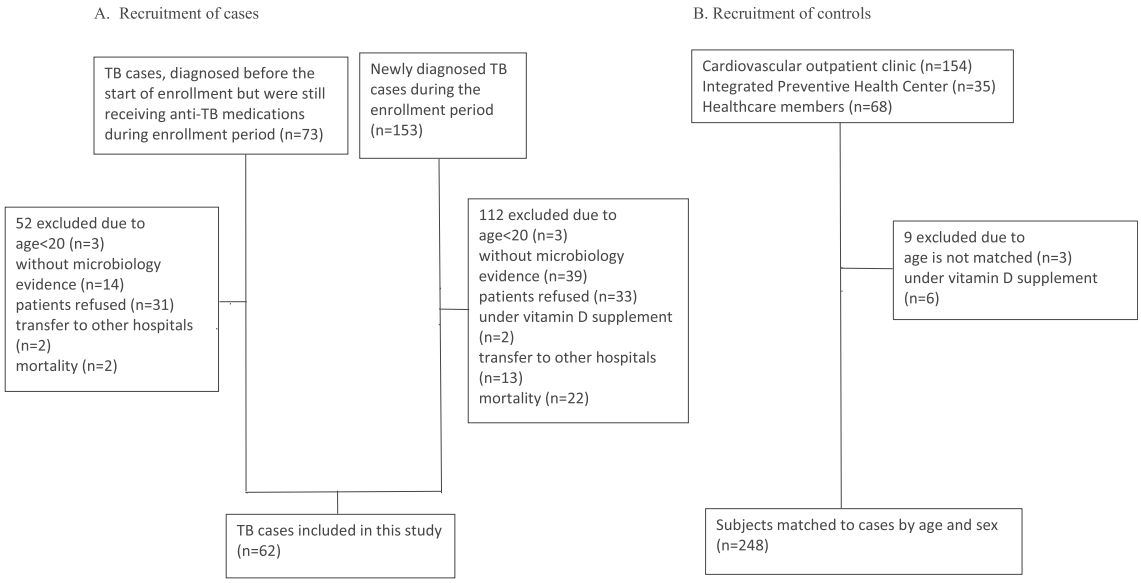

Fig. 1. Enrollment of participants (A) TB cases and (B) control cases.

Table 1 Characteristics of sex-and age-matched TB cases and controls.

	Cases n = 62 (%)	Controls n = 248 (%)	*p*-value
Age (years)	58.5 ± 14.3 (21–88)	58.6 ± 14.5 (20–89)	0.955
Male sex	44 (71%)	176 (71%)	1.0
Body-mass index (kg/m^2)	22.9 ± 3.3 (14.7–30.1)	25.2 ± 3.9 (16.2–40.7)	<0.001
Residence in Taiwan	North (100%)	North (100%)	1.0
Diabetes mellitus	16 (25.8%)	55 (22.2%)	0.54
Steroid use	0 (0%)	2 (0.8%)	0.48
End stage renal disease	0 (0%)	1 (0.4%)	1.0
Liver cirrhosis	3 (4.8%)	2 (0.8%)	0.056
Congestive heart failure	2 (3.2%)	16 (6.5%)	0.330
Smoking	22 (35.5%)	43 (17.3%)	0.002
Alcohol use behavior	3 (4.8%)	1 (0.4%)	0.026
25(OH)D level (ng/ml)	21.25 ± 8.93 (5.3–45.8)	24.45 ± 8.36 (6.3–52.2)	0.008
25(OH)D < 20 ng/ml	32 (51.6%)	74 (29.8%)	0.001

n, number.

Characteristics of cases versus controls

In univariable analysis, BMI was significantly lower in people with TB (22.9 ± 3.3 kg/m^2) than that in control group (25.2 ± 3.9 kg/m^2) ($p < 0.001$) (Table 1). People with TB had a significantly higher proportion of smokers (35.5% vs 17.3%, $p = 0.002$), alcohol use behavior (4.8% vs 0.4%, $p = 0.026$), and VDD (n = 32, 51.6% vs n = 74, 29.8%, $p = 0.001$). The mean 25(OH)D concentration was significantly lower in people with TB than that in control group (21.25 ± 8.93 ng/ml vs 24.45 ± 8.36 ng/ml, $p = 0.008$). There was a borderline significance of higher proportion of liver cirrhosis among people with TB (4.8%) compared to control group (0.4%) ($p = 0.056$). However, there were no significant differences in other underlying diseases such as diabetes mellitus (25.9% vs 22.2%, $p = 0.54$), steroid use (0% vs 0.8%, $p = 0.48$), end-stage renal disease (0% vs 0.4%, $p = 1.0$), and congestive heart failure (3.2% vs 6.5%, $p = 0.33$).

TB patients with serum vitamin D level < 20 ng/ml versus ≥20 ng/ml at enrollment

In people with TB, we compared the clinical characteristics of 32 people with VDD (25(OH)D concentration 14.34 ± 3.79 ng/ml), including four (6.5%) had severe VDD with levels below 10 ng/ml, and that of the 30 people without VDD (25(OH)D concentration 28.62 ± 6.6 ng/ml) (Table 2). Both VDD and non-VDD groups had around half of people with positive AFB stain in sputum (55% vs 53%, respectively, $p = 0.622$), and most of them had high grade 4+ (41% vs 33%, $p = 0.647$). Besides, nearly 60% of both

Table 2 Comparison of vitamin D level <20 and ≥ 20 ng/ml in TB patients.

	Vitamin D concentration <20 ng/ml, n = 32 (%)	Vitamin D concentration ≥20 ng/ml, n = 30 (%)	*p*-value
Vitamin D concentration (ng/ml)	14.34 ± 3.79 (5.3–19.9)	28.62 ± 6.6 (20~45.8)	<0.0011
Age (years)	56.66 ± 16.20 (29–88)	60.47 ± 11.89 (21–80)	0.298
Male	23 (72%)	21 (70%)	0.871
BMI (kg/m^2)	22.37 ± 3.46 (14.72–29.23)	23.41 ± 3.08 (17.89–30.05)	0.22
Diabetes mellitus	9 (28%)	6 (20%)	0.455
Pulmonary TB	31 (97%)	28 (93.3%)	0.607
Sputum AFB stain positive /grading in 4+	17 (55%) /n = 7, 41%	15 (53%) /n = 5, 33%	0.622/0.647
Cavitation on CXR /multiple lobes	18 (58%) /10 (55.6%)	16 (57%) /5 (31.3%)	0.818/0.154
Negative conversion of sputum culture (days)	37.57 ± 22.26 (n = 28[a], 6-148)	42.92 ± 34.35 (n = 25[b], 13-109)	0.5
Negative conversion of sputum AFB (days)	33.25 ± 18.23 (n = 16[c], 10-55)	75.21 ± 100.08 (n = 14[d], 6-360)	0.145

[a] Three patients' sputum culture didn't have microbiology evidence and the TB was diagnosed by lung tissue biopsy in two patients and bronchial washing fluid in one patient.
[b] Three patients' sputum culture didn't have microbiology evidence and the TB was diagnosed by lung tissue biopsy in two patients and bronchial washing fluid in one patient.
[c] One patient's sputum AFB was not yet negative conversion.
[d] One patient's sputum AFB was not yet negative conversion.

Table 3 Comparison of TB patients received anti-TB medications ≤30 days versus >30 days at the time of vitamin D concentration measurement.

	≤30 days n = 22 (%)	>30 days n = 40 (%)	*p*-value
Duration of anti-TB therapy when vitamin D was measured (days)	10.5 ± 7.87 (0–21)	100.86 ± 45.45 (36–370)	<0.001
Age (years)	58.77 ± 13.9 (37–85)	58.4 ± 14.7 (21–88)	0.912
Male	16 (73%)	28 (70%)	1
Pulmonary tuberculosis	21[a] (95.5%)	38[b] (95%)	1
BMI (kg/m^2)	22.13 ± 3.73 (14.7–30.1)	23.3 ± 3.01 (16.2–40.7)	0.188
Vitamin D concentration (ng/ml)	21.96 ± 8.84 (11.1–45.8)	20.86 ± 9.06 (5.3–37.7)	0.647
Case No. Of vitamin D concentration <20 ng/ml	11 (50%)	21 (52.5%)	0.851

[a] One patient was diagnosed of neck TB lymph adenopathy.
[b] Two patients were not pulmonary TB, including one had TB meningitis and one had neck TB lymph adenopathy.

groups had cavitation on CXR (58% vs 57%, $p = 0.818$). Half of people with VDD (55.6%) had multiple lobar involvement; while only 31.3% of people without VDD had the same findings ($p = 0.154$). Further, in people with TB, the duration of negative conversion of sputum, either culture or AFB, didn't differ significantly between people with or without VDD ($p = 0.5$ and $p = 0.145$, respectively).

TB patients received anti-TB medications ≤30 days versus >30 days at enrollment

All TB patients who accepted anti-TB therapy in the study received the same regimen, which included an intensive phase of 2 months (isoniazid, rifampin, ethambutol, and pyrazinamide), followed by a continuation phase of either 4 or 7 months. Twenty-two patients in TB group (22/62, 35.5%) had their 25(OH)D measured within 30 days (10.5 ± 7.87 days) of the initiation of the anti-TB regimens; the other 40 patients had their 25(OH)D measured after being treated for more than one month with anti-TB regimens (100.86 ± 45.45 days) ($p < 0.001$) (Table 3). However. There was no difference in the measured 25(OH)D levels between those who received anti-TB drugs for less or more than 30 days (21.96 ± 8.84 ng/ml vs 20.86 ± 9.06 ng/ml, $p = 0.647$). Besides, there was no association between the serum vitamin D concentration and the duration of anti-TB treatment (Pearson's correlation coefficient $r = -0.013$, $p = 0.92$) (supplemental data 1).

Risk factors for active TB

In multivariable analysis, VDD (adjusted odds ratio [aOR]: 3.03, 95% confidence interval [CI]: 1.51–6.10, $p = 0.002$), lower BMI (aOR: 0.81, 95% CI: 0.72–0.90, $p < 0.001$), liver cirrhosis (aOR: 8.99, 95% CI: 1.09–74.34, $p = 0.042$), and cigarette smoking (aOR: 4.52, 95% CI: 1.85–11.00, $p = 0.001$) were independent risk factors for incident active TB (Table 4). In Taiwan, VDD was not a good indicator of undernutrition, since there existed only a low correlation between serum vitamin D level and BMI (Pearson's correlation coefficient $r = 0.133$, $p = 0.019$, supplemental data 2). Moreover, there was a linear rather than U-shape trend between 25(OH)D concentration and the risk of incident active TB in people with severe VDD (<10 ng/ml, aOR 3.74, 95% CI 1.17–11.97), moderate VDD (10–20 ng/ml, aOR 1.63, 95% CI 0.80–3.35), vitamin D insufficiency (20–30 ng/ml, aOR 1.06, 95% CI 0.49–2.27) and vitamin D sufficiency (≥30 ng/ml, the reference) (supplemental data 3). Overall, the absolute 25(OH)D level (ng/mL) was inversely associated with the risk of incident active TB (aOR: 0.95, 95% CI: 0.91–0.99) (supplemental data 4).

Table 4 Multivariable analysis of risk factors for active TB diseases.

	Adjusted odd ratio	95% confidence interval	*p*-value
Ratio of 25(OH)D level <20 ng/ml	3.03	1.51–6.10	0.002
Body mass index	0.81	0.72–0.90	<0.001
Liver cirrhosis	8.99	1.09–74.34	0.042
Smoking	4.52	1.85–11.00	0.001
Alcohol use behavior	7.23	0.51–98.40	0.138

Discussion

Our data provided new evidence on the association between VDD and incident active TB among non-HIV people. We observed an overlapping distribution of 25(OH)D levels between people in TB and control group, with a statistically significant difference (21.25 ± 8.93 vs 24.45 ± 8.36 ng/ml, $p = 0.008$). However, after adjusting for the confounders, including BMI, smoking, alcohol use behavior, and liver cirrhosis, the same data showed that severe to moderate VDD (25(OH)D level <20 ng/ml) was associated with a three-fold increased risk of incident active TB (aOR: 3.03, 95% CI: 1.51–6.10, $p = 0.002$). Our results highlight that controlling the effects of major confounding factors unmasks the important role of VDD as a risk factor for incident active TB.

Our study found that vitamin D level did not alter significantly between people with TB treated with anti-TB drugs ≤30 days and those treated >30 days (21.96 ± 8.84 vs 20.86 ± 9.06 ng/ml, $p = 0.647$). The same findings were reported in a meta-analysis, which revealed that anti-TB treatment did not affect 25(OH)D levels, and people with TB who underwent the complete TB treatment course still

had lower 25(OH)D level, compared with people in the control group.[10] These observations further suggest that VDD is a risk factor rather than a consequence of active TB disease.

Our data confirm that low BMI is a risk factor for TB. Chronic undernutrition is prevalent in low-to middle-income countries, such as sub-Saharan Africa and Southern Asia, where TB is endemic due to the secondary immunodeficiency attributed to undernutrition.[18] BMI is a common indicator to measure nutritional status, and lower BMI has been found to be associated with TB in several previous studies. In India, Jaimni V et al. reported that people with TB had a significantly lower BMI than people in the control group (19.4 kg/m^2 vs 24.0 kg/m^2, $p < 0.0001$).[19] In Korea, Hong JY et al. noted the same finding that BMI of people with TB was lower than the control group (20.65 kg/m^2 vs 23.65 kg/m^2, $p < 0.001$).[20] Our study also showed that a lower BMI in people with TB than the control group (22.9 kg/m^2 vs 25.2 kg/m^2, $p < 0.001$). Based on the association between low BMI and TB, Bueno H et al. suggested that increasing a 2600 kcal/day diet for adults with a BMI of 16–18.4 kg/m^2 might be a cost-effective strategy in reducing TB incidence and mortality in India.[21] Impaired phagocytosis, antigen presentation, activation of Th1 response, and granulomatous formation processes against *M. tuberculosis* had been suggested as the mechanisms behind the association between undernutrition and TB.[18]

Dheda K et al. (2016) reviewed evidence supporting that smoking is a risk factor for TB in The Lancet.[22] Our study confirms the negative impact of cigarette smoking on the risk for developing active TB. Wang EY et al. reported that smoking is associated with delayed negative conversion and unfavorable treatment outcomes in a meta-analysis.[23] Khan AH et al. concluded that the smokers had a higher risk for mortality and treatment failure in people with TB in Malaysia.[15] Therefore, smoking cessation program is indicated in people with TB. Our study shows a strong association between smoking and TB (aOR 4.52, $p = 0.001$), indicating the need to control cigarette smoking in analyzing the negative effect of VDD on risk with active TB.

Our study verifies that liver cirrhosis is another risk factor for developing active TB. A nationwide epidemiological study conducted in Taiwan from 1998 to 2007 revealed that 2.32% of patients with cirrhosis developed TB, a percentage significantly higher than the 0.46% observed in individuals without cirrhosis ($p < 0.001$).[24] Another study from India showed a 15 times higher prevalence rate of TB in people with liver cirrhosis than general population.[25] People with liver cirrhosis may have reticuloendothelial system dysfunction, resulting in increased susceptibility to *M. tuberculosis*.[26] Our data show a significant association between liver cirrhosis and TB (aOR 8.99, $p = 0.042$), which indicates that liver cirrhosis needs to be controlled in analyzing the association between VDD and active TB disease.

Poorly controlled diabetes mellitus is considered a risk factor for TB with a two-to four-fold increased risk of active TB compared to patients without diabetes.[27] The impaired immune response in diabetes increases the susceptibility to *M. tuberculosis* infection.[28] In our study, both people with TB and people in the control group had a similar prevalence of diabetes mellitus (25.8% vs 22.2%, $p = 0.54$). It should be noted that the majority (60%) of individuals in the control group were enrolled from cardiovascular outpatient clinics, where 27.7% of participants were diagnosed with diabetes mellitus. This relatively higher proportion of individuals with diabetes in the control group could potentially account for the lack of association observed in our study regarding diabetes as a risk factor for active TB. Given that patients with cardiovascular diseases have a higher prevalence of diabetes compared to the general population, our study may underestimate the actual impact of diabetes on the incidence of active TB disease.

In India, Jaimni V et al. reported that TB patients with VDD had more severe symptoms, higher sputum smear positivity, and extensive lesions on CXR, compared with TB patients without VDD.[19] Though the sample size was not powered to compare differences in TB severity, our data showed a higher bacterial load (AFB 4+: 41% vs 33%, $p = 0.647$) and more multiple lobar cavitations in CXR (55.6% vs 31.3%, $p = 0.154$) in TB patients with VDD than TB patients without VDD. Further studies with larger sample size would be required to confirm these findings.

Our study has the following limitations. First, the participants in this study were enrolled in a single medical center in northern Taiwan. Nevertheless, VDD prevalence among participants in our study (34.2%), is similar to worldwide surveillance results which reports that VDD is a common health problem, with 37.3% of the global population having a serum level below 20 ng/ml.[29] The other TB risk factors identified in our study (low BMI, cigarette smoking, and liver cirrhosis) are also consistent with literature worldwide. Therefore, our study might still have a good external validity. Second, we did not enroll HIV-positive subjects, and none of the enrolled TB patients had multidrug resistant (MDR) TB. Therefore, our findings may not be generalizable to HIV-positive population and MDR-TB. Third, a high proportion of subjects (60%) in the control group were enrolled from ambulatory patients regularly followed up at outpatient cardiovascular clinics. However, current evidence shows that cardiovascular diseases patients are more likely to have VDD compared with general population.[30] Therefore, our study may underestimate the association between VDD and incident active TB disease. Fourth, our study did not incorporate a detailed inquiry into the food intake, socio-economic status, and educational levels of the enrolled subjects, factors that could confound the serum levels of 25(OH)D. Fifth, the enrolled cases did not undergo repeat testing. Nevertheless, our study revealed that patients who received anti-TB treatment for more or less than 30 days did not show a significant difference in serum 25(OH)D levels, though the small number of cases is a concern. Further studies are required to explore the role of vitamin D in the prognosis and outcomes of pulmonary TB. Finally, although Taiwan is an endemic area for *M. tuberculosis* infection, current data do not support an association between serum vitamin D levels and the incidence of latent TB.[31,32]

In conclusion, our study added new data to support that vitamin D deficiency is an independent risk factor for incident TB diseases among non-HIV patients after accounting for the effect of important confounders including low BMI, cigarette smoking, and liver cirrhosis, which could be behind the high heterogeneity observed in meta-analyses of

observational studies. Future randomized trials examining the effect of vitamin D supplementation on TB incidence should focus on people with a low BMI or other risk factors to maximize the statistical power.

Author contributions

Meng-Shiuan Hsu: conceptualization, methodology, investigation, validation, statistical analysis; visualization, writing — original draft, writing — review and editing, and funding acquisition; Tzu-Chien Chung: statistical analysis; Shih-Lung Cheng: cases enrollment and chest image reading; Ping-Huai Wang: cases enrollment; Yen-Wen Wu, Jung-Cheng Hsu, Bing-Hsiean Tzeng, Heng-Hsu Lin, Chung-Ming Tu: controls enrollment; Fang-Yeh Chu: measurement of vitamin D level; Chi-Tai Fang conceptualization, methodology, statistical analysis; supervision, writing — original draft, writing — review and editing, project administration, and funding acquisition; All authors critically reviewed and approved the submitted version of the manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jmii.2024.03.005.