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ORIGINAL ARTICLE

Severe vitamin D deficiency is associated with non-tuberculous mycobacterial lung disease: A case-control study

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ABSTRACT

Background and objective: **Previous studies have found evidence of an association between tuberculosis and vitamin D deficiency (VDD). However, the association between VDD and infection caused by non-tuberculous mycobacteria (NTM) has never been studied. This study evaluated the prevalence and severity of VDD in NTM lung disease and attempted to identify predictive factors.**

Methods: **Age- and sex-matched case-control study was conducted to assess the prevalence and severity of VDD in patients with NTM lung disease.**

Results: **After adjusting for potential confounding factors, the adjusted mean serum level of 25-hydroxyvitamin D (25(OH)D) levels was lower in 104 patients with NTM lung disease (10.7 ng/mL, 95% confidence interval (CI) 4.5–16.8 ng/mL) than that of 312 controls (13.7 ng/mL, 95% CI 7.4–19.5 ng/mL) (P = 0.012). Although the prevalence of VDD defined as serum 25(OH)D level <20 ng/mL was not different, severe VDD defined as serum 25(OH)D level <10 ng/mL was more prevalent in patients (P < 0.001). In multivariate analysis, severe (but not mild) VDD was independently associated with NTM lung disease (adjusted odds ratio 3.9, 95% CI 1.9–8.5, P < 0.001).**

Conclusions: **Patients with NTM lung disease have a high prevalence of severe VDD and VDD was independently associated with NTM lung disease. Further studies are needed to examine causality.**

Key words: case-control study, Korea, *Mycobacterium avium* complex, non-tuberculous mycobacteria, vitamin D.

Abbreviation: BMI, body mass index; CI, confidence interval; NTM, non-tuberculous mycobacteria; VDD, vitamin D deficiency.

SUMMARY AT A GLANCE

Many epidemiological studies have found evidence of an association between tuberculosis and vitamin D deficiency (VDD). However, the association between VDD and infection caused by non-tuberculous mycobacteria (NTM) has never been studied. This case-control study showed that patients with NTM lung disease have a high prevalence of severe VDD.

INTRODUCTION

The incidence of lung disease caused by non-tuberculous mycobacteria (NTM) in human immunodeficiency virus-negative patients has been increasing worldwide.[1,2] A substantial proportion of these patients have no predisposing risk factors.[1] *Mycobacterium avium* complex and *M. abscessus* complex are well-known organisms that cause these forms of NTM lung disease.[3–8]

NTM are ubiquitous in the environment, and exposure to these organisms is inevitable. However, disease is rare, which suggests that normal host defence mechanisms are effective enough to prevent infection.[9] Therefore, otherwise healthy individuals who develop NTM lung disease may have susceptibility factors that make them vulnerable to infections.[10,11]

Vitamin D has a critical role in the innate immune system and appears to have an important role in protection against respiratory tract infections.[12] Vitamin D can play an important role as an immunomodulator of the innate immune response against *M. tuberculosis*.[13,14] Many epidemiological studies have found evidence of an association between 25(OH) vitamin D (25(OH)D) levels and tuberculosis,[15–19] although some discrepancies exist among studies.[20–22] However, the association between vitamin D deficiency (VDD) and NTM infections has never been studied.

In this case-control study, we investigated the prevalence and severity of VDD in patients with NTM lung disease and compared them with controls sourced from the fourth Korean National Health and Nutrition Examination Survey in 2008.[23]

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METHODS

Consecutive patients with the nodular bronchiectatic form of NTM lung disease who were diagnosed between January 2008 and December 2008 were enrolled in an ongoing prospective observational cohort study to investigate NTM lung disease (ClinicalTrials.gov Identifier: NCT00970801) at the Samsung Medical Center (a 1961-bed referral hospital in Seoul, Korea). All of the patients met diagnostic criteria for NTM lung disease according to the guidelines of the American Thoracic Society[1] and had characteristic findings on high-resolution computed tomography scans.[24,25] The patients with concurrent pulmonary tuberculosis were excluded. In addition, immunocompromised patients were excluded. The diagnosis was based on culture positivity from at least two separate expectorated sputum samples in 86 patients (83%) and on bronchial washing or bronchoalveolar lavage fluid culture positivity in the remaining 18 patients (17%). Sputum smears and mycobacterial cultures were performed with standard methods as described.[26] NTM species were identified using a polymerase chain reaction and restriction length polymorphism method based on the *rpoB* gene and confirmed using multilocus sequence analysis based on *hsp65*, *rpoB* and 16S ribosomal RNA fragments, as described previously.[27–30] The aetiological organisms were *M. avium* complex in 69 (66%) patients (*M. avium* in 38 and *M. intracellulare* in 31) and *M. abscessus* complex in 35 (34%) patients (*M. abscessus* in 14 and *M. massiliense* in 21). The study was approved by the Institutional Review Board at our institution, and written informed consent was obtained from each participant.

Peripheral blood was collected before the initiation of antibiotic treatment, and serum was aliquoted and stored at –80°C until analysis. The serum 25(OH)D levels were measured in duplicate using an enzyme-linked immunosorbent assay kit (Immundiagnostik AG, Bensheim, Germany) following the manufacturer's instructions. VDD was defined as a serum 25(OH)D level of less than 20 ng/mL.[31] As a serum 25(OH)D level for VDD can be defined as either 10 or 20 ng/mL depending on the level that is defined as normal,[31,32] we classified VDD as mild VDD, for a serum 25(OH)D level of 10–19 ng/mL, or severe VDD, for a serum 25(OH)D level <10 ng/mL.

The control group was randomly selected from the study group of the fourth Korean National Health and Nutrition Examination Survey,[23] and was matched in a 1:3 ratio for sex and age to ensure an adequate sample size for detecting a 20% of difference with 95% of chance. Serum 25(OH)D data were available for 3047 males and 3878 females aged 10 years and older.[23]

The data were compared using the Mann–Whitney *U*-test for the continuous variables and a chi-squared or Fisher's exact test for categorical variables. A multiple linear regression model was used to adjust for potential confounding factors in the relationship between NTM lung disease and serum 25(OH)D level. We adjusted for age, sex, body mass index (BMI), previous history of tuberculosis, smoking history and comorbidities, including diabetes, chronic liver disease and chronic kidney disease that have been linked to an increased prevalence of VDD.[31] In addition, multiple logistic regression model was used to adjust for potential confounding factors described earlier in the association between the severity of VDD and the development of NTM lung disease, as measured by the estimated odds ratio with a 95% confidence interval (CI).

To identify independent predicting factors associated with VDD and severe VDD in patient with NTM lung disease, multiple logistic regression analysis was used. Variables with *P*-values <0.25 in the univariable analysis, and the a priori variables of age, sex, BMI and previous history of tuberculosis were entered into a multiple logistic regression model in which VDD was the outcome variable. All tests were two-sided, and a *P*-value <0.05 was considered significant. Data were analysed using IBM SPSS Statistics 19.0 (IBM, Chicago, IL, USA).

RESULTS

The baseline characteristics of the patients with NTM lung disease and controls are presented in Table 1. BMI among patient with NTM lung disease (median

Table 1 Baseline characteristics of the study participants

Variables	NTM patients ($n = 104$)	Controls ($n = 312$)	*P*-value
Age, years	57 (50–65)	57 (50–65)	NA
Gender, male	23 (22)	69 (22)	NA
Body mass index (kg/m^2)	20.6 (19.5–22.3)	23.9 (21.8–25.9)	<0.001
Current smoker	1 (1)	40 (13)	<0.001
Previous history of tuberculosis	35 (34)	38 (12)	<0.001
Comorbidity			
Diabetes	7 (7)	30 (10)	0.358
Chronic heart disease	12 (12)	8 (3)	0.001
Chronic liver disease	7 (7)	4 (1)	0.007
Chronic kidney disease	1 (1)	1 (0)	0.441

Values are expressed as medians (interquartile range) or frequencies (%).
NA, not applicable; NTM, non-tuberculous mycobacteria.

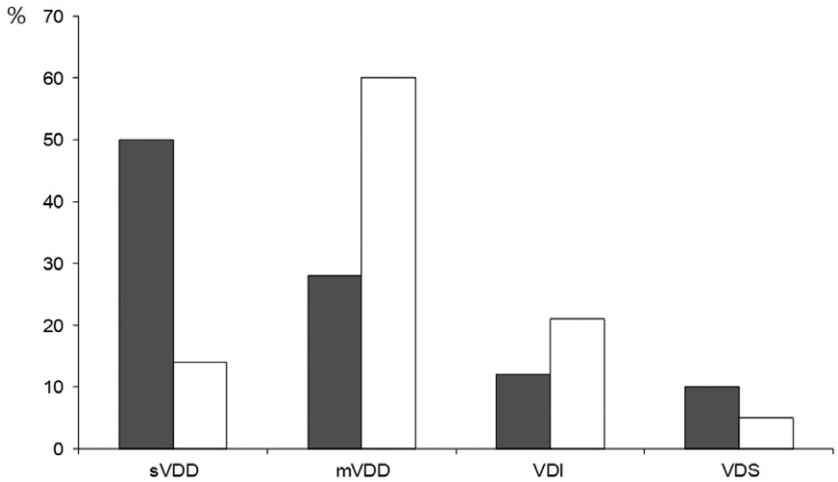

Figure 1 Prevalence of different degrees of vitamin D deficiency (VDD) among cases with non-tuberculous mycobacterial lung disease and controls. The serum 25(OH)D levels were classified as follows:[31] severe VDD (sVDD): less than 10 ng/mL; mild VDD (mVDD): 10–19 ng/mL; vitamin D insufficiency (VDI): 21–29 ng/mL; and vitamin D sufficiency (VDS): 30 ng/mL or greater. (■) cases; (□) controls.

Table 2 Multiple linear regression for potential explanatory variables of serum 25(OH)D level

Variables	Coefficient (β)	95% CI	*P*-value
Age, years	0.098	0.024 to 0.171	0.009
Gender, male	–0.301	–2.348 to 1.746	0.773
Body mass index (kg/m^2)	–0.070	–0.332 to 0.193	0.603
Current smoker	–0.299	–3.160 to 2.562	0.837
Previous history of tuberculosis	–2.150	–4.361 to 0.060	0.057
Diabetes	–1.994	–4.892 to 0.904	0.177
Chronic liver disease	–0.540	–5.721 to 4.640	0.838
Chronic kidney disease	–2.984	–14.843 to 8.874	0.621
NTM lung disease	–2.703	–4.822 to –0.584	0.013

CI, confidence interval; NTM, non-tuberculous mycobacteria.

20.6 kg/m^2, interquartile range 19.5–22.3 kg/m^2) was significantly lower than that among controls (median 23.9 kg/m^2, interquartile range 21.8–25.9 kg/m^2, $P < 0.001$). The prevalence of current smoking was significantly higher in controls (13%) than in patients with NTM lung disease (1%). Previous history of tuberculosis was more common in patients with NTM lung disease (34%) than in controls (12%, $P < 0.001$). In addition, other comorbidities were also more common in the patients (Table 1).

The median 25(OH)D level was 9.8 ng/mL (interquartile range 4.6–18.6 ng/mL) in patients with NTM lung disease versus 15.4 ng/mL in controls (interquartile range 11.9–20.2 ng/mL, $P < 0.001$). Using the criterion of <20 ng/mL, the prevalence of VDD overall was not significantly different between the patients (78%) and controls (74%, $P = 0.433$). However, the prevalence of severe VDD was greater among patients (50%) than among the controls (14%, $P < 0.001$, Fig. 1). On the contrary, the prevalence of mild VDD was lesser among patient (28%) than among the controls (60%, $P < 0.001$, Fig. 1). The prevalence of vitamin D insufficiency and vitamin D sufficiency between two groups was not statistically different ($P = 0.060$ and $P = 0.094$, respectively).

The results of multivariate analysis with the multiple linear regression model are presented in Table 2. After adjusting for a priori variables of age, sex, BMI, previous history of tuberculosis, smoking history and comorbidities, including diabetes, chronic liver disease and chronic kidney disease, the adjusted mean of 25(OH)D levels was lower in patients with NTM lung disease (10.346 ng/mL, 95% CI 4.167–16.526 ng/mL) than that of the controls (13.049 ng/mL, 95% CI 6.986–19.112 ng/mL) ($P = 0.013$).

The results of univariate and multivariate analyses with multiple logistic regression model are presented in Table 3. After adjusting for a priori variables of age, sex, BMI, previous history of tuberculosis, smoking history and comorbidities, including diabetes, chronic liver disease and chronic kidney disease, severe VDD was independently associated with NTM lung disease (adjusted odds ratio 4.1, 95% CI 1.9–8.8, $P < 0.001$). However, mild VDD was not associated with NTM lung disease (adjusted odds ratio 0.6, 95% CI 0.3–1.3, $P = 0.186$).

Univariable comparisons of the baseline characteristics of the 104 patients with NTM lung disease according to VDD status are presented in Table S1 in the online supporting information. There was no significant difference in age, BMI or previous history of tuberculosis. However, male gender was more common among patients with VDD (22/81, 27%) than among patients without VDD (1/23, 4%, $P = 0.022$).

Table 3 Univariable and multivariable analysis with logistic regression model for probability of non-tuberculous mycobacterial lung disease

Variables	Univariable analysis		Multivariable analysis	
	OR (95% CI)	*P*-value	Adjusted OR (95% CI)	*P*-value
Age, years	1.000 (0.980–1.020)	1.000	1.006 (0.979–1.033)	0.663
Male gender	1.000 (0.586–1.707)	1.000	0.605 (0.278–1.318)	0.206
Body mass index (kg/m^2)	0.650 (0.584–0.724)	<0.001	0.658 (0.582–0.745)	<0.001
Current smoker	0.065 (0.009–0.481)	0.007	0.012 (0.001–0.153)	0.001
Previous history of tuberculosis	3.617 (2.130–6.145)	<0.001	1.969 (1.002–3.867)	0.049
Diabetes	0.671 (0.286–1.577)	0.360	0.597 (0.174–2.043)	0.411
Chronic liver disease	5.503 (1.577–19.197)	0.007	16.733 (2.152–130.126)	0.007
Chronic kidney disease	2.990 (0.185–48.238)	0.440	0.395 (0.008–18.771)	0.637
Vitamin D deficiency				
Mild deficiency	0.543 (0.296–0.996)	0.048	0.620 (0.305–1.259)	0.186
Severe deficiency	4.259 (2.304–7.874)	< 0.001	4.105 (1.922–8.770)	< 0.001

CI, confidence interval; OR, odds ratio.

The proportion of subjects with *M. avium* complex lung disease was higher among patients with VDD (59/81, 73%) than among patients without VDD (10/23, 44%, $P = 0.009$). After adjusting for potential confounding factors, *M. avium* complex was significantly associated with VDD (adjusted odds ratio 3.5, 95% CI 1.2–9.7, $P = 0.025$) (Table S2 in the online supporting information). Predicting factors associated with severe VDD were also evaluated. However, there was no independent predicting factor associated with severe VDD, although there was a trend of that *M. avium* complex lung disease was more common in patients with severe VDD (adjusted odds ratio 2.441, 95% CI 0.970–6.143, $P = 0.058$).

DISCUSSION

To our knowledge, this is the first study to evaluate the prevalence and severity of VDD in patients with NTM lung disease. The results of our case-control study indicate that the median 25(OH)D levels for patients with NTM lung disease are significantly lower than are those of healthy controls. Although the prevalence of VDD overall was not significantly different, severe VDD was more prevalent in patients with NTM lung disease than among controls. Additionally, we found that *M. avium* complex infection was independently associated with VDD in patients with NTM lung disease.

The serum 25(OH)D level is the best indicator of overall vitamin D status because this measurement reflects total vitamin D from dietary intake and sunlight exposure.[31] Defining a level of serum 25(OH)D as deficient or insufficient depends on the level that is defined as normal, but currently, there is no consensus on the optimal serum level of 25(OH)D.[33] Because there are no standard cut-offs, the definition of VDD may affect estimates of its prevalence. Previously, according to the World Health Organization, levels below 10 ng/mL were considered deficient, and levels below 20 ng/mL were classified as insufficient.[32] When that range was used, the prevalence of VDD among our patients was 50%, which was significantly higher than the prevalence of 14% among healthy controls ($P < 0.001$). However, with the recent changes as in the laboratory reference ranges, VDD is defined by most experts as a serum 25(OH)D level of less than 20 ng/mL.[34–37] With the use of this definition, 47–64% of Korean men and women are deficient in vitamin D.[23] This high level of VDD in the general population might be a reason why the prevalence of VDD among our patients with NTM lung disease was not significantly different from the prevalence among the age- and sex-matched controls. However, the median level of serum 25(OH)D in patients with NTM lung disease was significantly lower than that in controls.

Although our data suggest a link between NTM lung disease and low serum 25(OH)D levels, there are many causes of VDD in the general population that are not related to NTM infection.[31] The prevalence of VDD is particularly high in the elderly due to an age-associated decline in cutaneous vitamin D production and decreased dietary vitamin D intake.[37] This bias was excluded by using age-matched controls in the comparison of serum 25(OH)D levels between the patients and the controls, and was adjusted by multivariate analysis of the predicting factors for VDD in the patients. In addition, BMI is inversely related to the serum 25(OH)D level.[37]

Many case-control studies and a prospective cohort study demonstrated that low vitamin D level is associated with pulmonary tuberculosis.[15–19] There are good evidences to suggest that VDD compromises cell-mediated immunity and increases susceptibility to mycobacterial infection.[12] Vitamin D is required for an interferon-γ-mediated pathway in macrophages that leads to autophagy, phagosomal maturation and other antimicrobial activities against *M. tuberculosis* infection.[38] However, some studies from Asian countries like Indonesia, China and Korea did not support the hypothesis that VDD predisposes to tuberculosis.[20–22] Discrepancies between these studies

may be due to the higher prevalence of VDD among Asians.[22]

There are several limitations to our study. Because this study was a case-control design, no causal inferences could be made for the observed association between VDD and NTM lung disease. Moreover, our study was conducted at a single institute with relatively small sample size, which may limit the generalizability of our findings. Second, the data on other causes of VDD including dietary intake, alcohol consumption, exposure to sunlight and medications,[31] which may have influenced the significance of our findings, were not obtained. Finally, we did not ascertain the seasons of blood sampling for 25(OH)D measurement among cases and controls.

In conclusion, this case-control study showed that patients with NTM lung disease have a high prevalence of severe VDD. Moreover, severe VDD was independently associated with NTM lung disease after adjustments for potential confounding factors. Studies are needed to clarify if there is a causal association between VDD and NTM lung disease.

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Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1 Univariable comparisons of baseline characteristics between 104 patients with non-tuberculous mycobacterial lung disease according to vitamin D deficiency (VDD).

Table S2 Univariable and multivariable analysis with logistic regression model for probability of vitamin D deficiency in patient with non-tuberculous mycobacterial lung disease.