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Original article

Low serum 25-hydroxyvitamin D level: An independent risk factor for tuberculosis?

Ji Hae Kim [a], Jong-Sun Park [b], Young-Jae Cho [b], Ho-Il Yoon [b], Jung Han Song [c], Choon-Tack Lee [b], Jae Ho Lee [b,*]

[a] College of Pharmacy and Research Institute of Pharmaceutical Sciences, Seoul National University, Seoul, Republic of Korea
[b] Division of Pulmonary and Critical Care Medicine, Department of Internal Medicine and Lung Institute, Seoul National University College of Medicine, Seoul National University Bundang Hospital, Seongnam-si, Gyeonggi-do, Republic of Korea
[c] Department of Laboratory Medicine, Seoul National University College of Medicine, Seoul National University Bundang Hospital, Seongnam-si, Gyeonggi-do, Republic of Korea

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SUMMARY

Background & aims: Vitamin D deficiency has been associated with an increased risk of tuberculosis (TB). Low serum vitamin D levels may also be associated with poor nutritional status in TB patients. Therefore, this study aimed at evaluating the association between low serum vitamin D level and TB, regardless of other nutritional factors.

Methods: Baseline serum 25-hydroxyvitamin D (25(OH)D3) levels in TB patients were measured before treatment and 1 year after treatment onset using liquid chromatography–tandem mass spectrometry, and were compared with 25(OH)D3 levels in controls. Nutritional parameters were also measured in all subjects.

Results: In total, 165 active pulmonary TB patients and 197 controls were included in the study. Significantly higher prevalence of 25(OH)D3 insufficiency (<20 ng/mL) and deficiency (<10 ng/mL) in TB patients was showed compared to controls. Serum 25(OH)D3 levels and nutritional parameters were significantly lower in untreated TB patients than in controls. One year after TB treatment onset, nutritional parameters significantly increased; however, serum 25(OH)D3 levels in TB patients showed no significant improvement compared to baseline.

Conclusions: These results suggest that a low serum 25(OH)D3 level might be a risk factor for TB, independent of nutritional status.

1. Introduction

Tuberculosis (TB) remains a major public health problem around the world. In 2011, there were an estimated 8.7 million new cases of TB were reported and 1.4 million people died from TB.[1] The incidence and progression of TB are influenced by various factors including HIV infection, malnutrition, diabetes, smoking, alcoholism, and socioeconomic status. Of these factors, malnutrition constitutes one of major risk factors for TB.[2] Some data have suggested that micronutrient deficiencies, including iron, vitamin D, and vitamin B_{12} deficiencies, also increase the risk of TB.[3,4] The association between vitamin D deficiency and increased risk of TB has been specifically examined[5] and explained by the noncalcemic physiological actions of vitamin D on the immune system.[6]

In TB disease, vitamin D may play anti-microbial and anti-inflammatory roles through modulating monocyte-macrophage activity and lymphocytes responses.[6] Accordingly, vitamin D deficiency would leave the host more susceptible to TB disease by compromising the humoral immune defense.[7]

Several previous studies have compared vitamin D serum levels between TB patients and healthy control subjects,[8,9] and have examined changes in vitamin D levels according to anti-TB treatment.[10,11] Despite some inconsistencies in the results, some studies have suggested the existence of a clinical correlation between vitamin D deficiency and increased TB risk.

On the other hand, a recent study has suggested that low vitamin D levels may be the manifestation of poor nutritional status in TB patients.[12] Since TB patients with malnutrition may also suffer

* Corresponding author. Division of Pulmonary and Critical Care Medicine, Seoul National University Bundang Hospital, 82 Gumi-ro 173 Beon-gil, Bundang-gu, Seongnam-si, Gyeonggi-do 463-707, Republic of Korea. Tel.: +82 31 787 7011; fax: +82 31 787 2204.
E-mail address: jhlee7@snubh.org (J.H. Lee).

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from micronutrient deficiency, a possible correlation between vitamin D deficiency and malnutrition in TB patients cannot be excluded.

However, direct causal relationships between vitamin D status, malnutrition, and TB have not been clearly described yet. Therefore, this study aimed at evaluating the role of vitamin D deficiency as a risk factor for TB, independent of other nutritional parameters.

2. Subjects and methods

2.1. Study design

This case control study examined changes in vitamin D levels in TB patients undergoing treatment. Serum vitamin D levels were classified as: insufficient (<20 ng/mL) and deficient (<10 ng/mL, the lower limit of normal range).[13]

2.2. Subjects

Patients consecutively presenting to the Seoul National University Bundang Hospital with active pulmonary TB were prospectively recruited to this study. Subjects were enrolled in the study over a 3-year period extending from 2008 to 2010.

Pulmonary TB diagnosis was classified as: definite, probable, or possible. Diagnosis was considered: definite, if a culture grew *Mycobacterium tuberculosis*, or if a polymerase chain reaction test for TB yielded positive results; probable, if the lesion exhibited both typical histological and radiological TB characteristics, and responded well to anti-TB medication; possible, if the lesion exhibited typical TB radiological features, and responded well to anti-TB medication. HIV co-infection was not routinely examined, since pulmonary TB has been rarely reported in HIV-positive patients in Korea.[14]

Patients were treated with first-line anti-TB drug therapy consisting of an HREZ regimen (isoniazid, rifampicin, ethambutol, and pyrazinamide) for the first 2 months, followed by an HRE regimen (isoniazid, rifampicin, and ethambutol) for 4 months, in accordance with the American Thoracic Society guidelines.[15]

Patients with multi-drug resistant TB were excluded from the study. Patients who had any other serious concomitant disease that could affect vitamin D metabolism, such as malabsorption, liver or renal disorders, diabetes, and malignancy, were also excluded.

Simultaneously, healthy control subjects with no history of TB or of other chronic illness were selected and matched by age, sex, and ethnic origin. The study protocols were approved by the Institutional Review Board at Seoul National University Bundang Hospital (Seongnam, South Korea). All participants received full information on the study's purpose and provided informed consent to participate in the study.

2.3. Blood sampling and laboratory tests

Serum samples were obtained between 8 and 9 AM after overnight fasting and stored at −70 °C following centrifugation. The first blood sample was collected prior to the onset of anti-TB chemotherapy in TB patients, and the second blood sample was collected 1 year after treatment onset, which corresponds to 6 months after anti-TB treatment completion. Blood samples were only collected once for control subjects.

In this study, vitamin D level was defined as a serum level of 25 hydroxycholecalciferol, (25(OH)D3), which is generally accepted as the barometer for vitamin D status due to its stability and long half-life. Serum 25(OH)D3 levels were determined using liquid chromatography–tandem mass spectrometry.[16] By this method, both 25(OH)D3 and 25(OH)D2 could be detected, but the 25(OH)D3 component was used in this study to determine vitamin D status. The internal standard used was 26, 27-hexadeuterium labeled 23-OH Vitamin D3. The BEH C18 column (2.1 × 50 mm, 1.7 μm) and Quattro Premier tandem mass spectrometer (Waters, USA) were used.

Blood analysis, measuring hemoglobin, albumin, and total cholesterol levels, was performed on all subjects to evaluate their nutritional status. Body weight and body mass index (BMI) were obtained through patient history or physical examination. With regard to the selection of biomarkers of nutritional status, the method chosen for determination of malnutrition and the definition of each parameter have been used in other studies of TB.[17,18]

2.4. Statistical analysis

Sample size was estimated for 80% power at a two-sided 5% significance level. Data were tested for normal distribution using the Kolmogorov–Smirnov test. An independent sample *t*-test was used to assess the difference between groups (patients vs. control subjects, female subjects vs. male subjects). A paired Student's *t*-test and McNemar's test were used to assess the difference between baseline data before treatment and follow-up data after treatment for each subject.

Multivariate correlation analysis was used to evaluate the relationship between parameters, using Pearson's correlation coefficient. McNemar's test was used to evaluate the distributions of sampling months and of low 25(OH)D3 prevalence. SPSS 15.0 software (Chicago, IL, USA) was used for all statistical analyses, and a P-value < 0.05 was considered statistically significant.

3. Results

3.1. Baseline characteristics

In total, 165 TB patients were enrolled in this study. The second assessment was completed by 101 (61.2%) of these patients, while blood samples could not be collected at the specified time for 64 of these patients. Additionally, a total of 197 healthy subjects matched by age and sex were enrolled as control subjects. All subjects were ethnic Koreans. TB patients and control groups were similar in age and sex. However, BMI was significantly lower in TB patients than that for control subjects. Characteristics of patients and control subjects are presented in Table 1.

3.2. Comparison of serum 25(OH)D3 levels between TB patients and control subjects

Serum 25(OH)D3 levels in TB patients were significantly lower (3.62 ng/mL difference) than that in control subjects ($p = 0.005$ by multiple analysis, $p < 0.001$ by *t*-test, Table 2 and Fig. 1).

The prevalence of low 25(OH)D3 level was higher in TB patients than in the control subjects. 25(OH)D3 insufficiency (<20 ng/mL) and deficiency (<10 ng/mL) was estimated at 83.0% and 44.2% of TB patients, compared to 65.5% and 10.7% of the control subjects, respectively (Table 2 and Fig. 2). Serum 25(OH)D3 levels in definite TB patients only were also lower than those in control subjects (13.5 ± 9.10 ng/mL vs. 18.7 ± 8.33 ng/mL, $p < 0.001$).

3.3. Changes in serum 25(OH)D3 levels according to TB patient treatment

Serum 25(OH)D3 levels at follow-up, 1 year after TB treatment onset, were not significantly different from baseline 25(OH)D3 levels prior to treatment (Table 2 and Fig. 1). Moreover, no