Original Articles/นิพนธ์ต้นฉบับ

# The Association of Vitamin D Level and Pulmonary Tuberculosis in Thailand

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## Abstract

**Background:** About ten millions of the world population have vitamin D deficiency. The associations between vitamin D deficiency and tuberculosis (TB) may be explained by an evidence for an immunoregulatory role of the vitamin. A previous study has shown that polymorphisms in vitamin D receptor are associated with TB infection in some population and some studies showed the association of vitamin D deficiency with pulmonary TB as the risk factor. In Thailand, there has been no prior study about the association between vitamin D deficiency and TB patients.

**Method:** Serum 25-hydroxycholecalciferol concentrations were measured in patients whose clinical history and laboratory tests suggesting of active pulmonary tuberculosis. We measured serum calcium, phosphate, creatinine, liver function test and recorded the symptoms. Demographic data such as chest X-ray finding, body weight and height were collected. Age, sex matched control population who were not diagnosed having pulmonary TB, did not have symptom suggesting TB and had normal chest X-ray was measured for serum 25-hydroxycholecalciferol concentrations. We excluded those who had chronic kidney disease, nephrotic syndrome, liver cirrhosis and who use antiretroviral, steroid, antiepileptic drugs and supplementary nutrition which might influence the vitamin D level.

**Results:** We collected 30 patients in TB group and 31 healthy adults for control group. The characteristic between groups was not different. We observed vitamin D below 40 ng per milliliter in 90% (27/30) of the TB patients and 100% of the healthy control group and there was no statistical significance between these two groups.

**Discussion:** There was no statistical significant difference between mean serum 25-hydroxycholecalciferol in patients with pulmonary TB and control group. As compared with previous studies, the number of patients with pulmonary tuberculosis who had vitamin D deficiency in this study was higher than in the previous studies. The previous studies showed that vitamin D deficiency is associated with higher risk of active tuberculosis, but our study did not show this association.

**Key words:** Vitamin D, pulmonary tuberculosis, Vitamin D deficiency

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## Background

About ten million of the world population have vitamin D deficiency. There are several causes of vitamin D deficiency such as sun block users, dark colour skin, old age, malabsorption, obesity, some drugs, liver failure, nephrotic syndrome, and chronic kidney disease[1].

The sources of vitamin D are from sunlight, foods and nutritional supplement. Vitamin D from any source is metabolized in liver to 25-hydroxyvitamin D, which can be used as an indicator of vitamin D deficiency. This 25-hydroxyvitamin D is further metabolized to 1, 25-hydroxyvitamin D in kidney which is regulated by serum parathyroid hormone, calcium and phosphorus[1].

In Thailand, the overall prevalence of active tuberculosis from chest radiography is 2.0%[2]. The risk factors for developing tuberculosis are persons who recently have contacted with active tuberculosis patients, who had particular underlying diseases that were associated with impaired host defense mechanisms such as pulmonary fibrosis, silicosis, AIDS, chronic kidney disease, diabetes mellitus, intravenous drug user, receiving immunosuppressive drugs, post kidney transplant, malnutrition and underweight.

The associations between vitamin D deficiency and tuberculosis (TB) may be explained by an evidence for an immunoregulatory role of this vitamin. Deficiency of protein, zinc and active metabolite of vitamin D could be the cause of impaired T cell function, including decrease of the Th1 cytokines interleukin-2 and interferon gamma production. In vitro studies have reported that vitamin D metabolites can activate antimycobacterial responses of human monocyte and macrophages, enhance phagocytosis and granuloma formation and modulate cytokine response in pulmonary tuberculosis[3]. The other studies showed that vitamin D inhibits transcription of tryptophan-aspartate containing coat gene (TACO) which is involved in the survival of TB in macrophages[4,5]. In TB infected mice, study has found increased concentration of TB and lung lesions in mice which have vitamin D deficiency[6]. In human studies, there are the associations between polymorphisms in vitamin D receptors and TB infection[7,8]. Other studies showed vitamin D deficiency as one of the risk factor of pulmonary TB infection[9-12]. In Thailand, there has been no prior study about vitamin D deficiency and its association with TB patients. In this report, we recorded levels of vitamin D in both control and TB groups to find the association of vitamin D deficiency and TB infection.

## Method

We enrolled patients between June 2008 and December 2008. Serum 25-hydroxycholecalciferol concentrations were measured in those who were presenting to pulmonary and general medicine outpatient clinics in Ramathibodi Hospital with clinical history and laboratory suggesting of active pulmonary tuberculosis (pulmonary TB). We measured serum calcium, phosphate, creatinine, liver function test and recorded the symptoms. Demographic data such as chest X-ray findings, body weight and height were collected. Age, sex matched control population not diagnosed having pulmonary TB, did not have symptom suggesting TB and have normal chest X-ray was measured for serum 25-hydroxycholecalciferol concentrations and demographic data were recorded.

The exclusion criteria in either pulmonary TB or control groups were those who had chronic kidney disease, nephrotic syndrome, liver cirrhosis and who used antiretroviral drugs, corticosteroid, antiepileptic

drugs and supplementary nutrition, which may influence the vitamin D level.

### Definition of pulmonary tuberculosis

Smear positive pulmonary tuberculosis was defined as two or more initial sputum smear positive for AFB or one sputum smear positive for AFB plus radiographic abnormalities consistent with active pulmonary TB as determined by a clinician or one sputum smear positive for AFB plus sputum culture positive for *M. tuberculosis*[13].

Smear negative pulmonary tuberculosis was defined as the patient who did not meet definition for smear positive TB (at least three sputum specimens negative for AFB, and radiographic abnormalities consistent with active pulmonary TB, and no response to a course of broad-spectrum antibiotics, and decision by a clinician to treat with a full course of antituberculosis chemotherapy)[13].

### Definition of vitamin D deficiency

Vitamin D deficiency is generally defined by a serum 25-hydroxycholecalciferol concentration of 20 ng per milliliter or less, 21 to 29 ng per milliliter can be considered to indicate a relative insufficiency of vitamin D, and a level of 30 ng per milliliter or greater can be considered to indicate sufficient vitamin D due to evidence of the association with parathyroid levels and intestinal calcium transport[1]. For this study, we defined severe deficiency as a serum concentration of 20 ng per milliliter, less severe deficiency as 21-39 ng per milliliter and adequate concentrations as above 40 ng per milliliter. We had classified subgroups of vitamin D deficiency to mild, moderate and severe deficiency as defined by serum 25-hydroxycholecalciferol concentration of < 20 ng per milliliter as severe deficiency, serum 25-hydroxycholecalciferol concentration of 20-30 ng per milliliter as moderate deficiency and 31-39 into mild deficiency group.

### Statistical analysis

Pearson chi-square was used to assess statistical differences in proportions between groups ($P < 0.05$). Student's t-test was used to assess differences of means between 2 groups when there was a normal distribution, and one sample of Kolmogorov-Smirnov test was used when nonparametric analysis was needed.

## Results

We collected data from 30 patients in TB group, (17 males and 13 females), and 31 healthy adults in control group (12 males and 19 females). The characteristics of the two groups are described in Table 1.

In TB group, 10 patients were smear positive pulmonary TB, 20 patients were smear negative pulmonary TB, 13 patients were culture positive and 3 patients did not send sputum for culture. In smear negative pulmonary TB, there were positive sputum cultures in 6 patients (one was obtained lung tissue culture by CT guided biopsy).

Characteristics of chest radiographic findings in TB group were nodular or mass (n=8, 25.8%), reticulonodular infiltration (n=5, 16.1%), patchy infiltration (n=5, 16.1%), cavity (n=3, 9.6%), interstitial infiltration (n=2, 6.4%), pleural effusion (n=2, 6.4%), hilar enlargement (n=1, 3.2%), miliary (n=1, 3.2%), fibrotic (n=1, 3.2%), bronchiectasis (n=1, 3.2%) and 4 patients had more than one abnormal finding (Table 2).

Symptoms at the presentation in TB group were fever, cough, weight loss and hemoptysis. 8 patients (26.7%) had fever, 12 patients (40%) had cough, 6 patients (20%) had weight loss, 2 patients (6.7%) had hemoptysis and 9 patients (30%) had no symptom at presentation (Table 3).

**Table 1** Patient characteristics of TB group and control group

| | Pulmonary TB (n = 30) | Control (n = 31) | *P*-value |
|---|---|---|---|
| Age (mean ± SD) | 54.7 ± 19.17 | 59.81 ± 13.85 | 0.241 |
| Male (n,%) | 15, 50 | 13, 41.9 | 0.314 |
| Sunblock usage (n,%) | 2, 6.66 | 1, 3.22 | 0.312 |
| BMI ($kg/m^2$) (mean ± SD) | 19.21 ± 2.92 | 25.51 ± 3.58 | 0.143 |
| Albumin (g/L) (mean ± SD) | 20.03 ± 3.14 | 25.61 ± 4.99 | 0.025 |
| Vitamin D level (ng/ml) (mean ± SD) | 21.47 ± 7.14 | 20.74 ± 3.60 | 0.342 |

**Table 2** Chest radiographic findings in 30 patients of TB group

| Type of chest radiographic finding | n (%) |
|---|---|
| Nodular or mass | 8 (25.8) |
| Reticulonodular infiltration | 5 (16.1) |
| Patchy infiltration | 5 (16.1) |
| Cavitation | 3 (9.6) |
| Interstitial infiltration | 2 (6.4) |
| Pleural effusion | 2 (6.4) |
| Hilar enlargement | 1 (3.2) |
| Miliary | 1 (3.2) |
| Fibrotic | 1 (3.2) |
| Bronchiectasis | 1 (3.2) |

**Tabel 3** Symptoms at presentation in 30 patients of TB group

| Symptoms at presentation | n (%) |
|---|---|
| Fever | 8 (26.7) |
| Cough | 12 (40) |
| Weight loss | 6 (20) |
| Hemoptysis | 2 (6.7) |
| No symptom | 9 (30) |

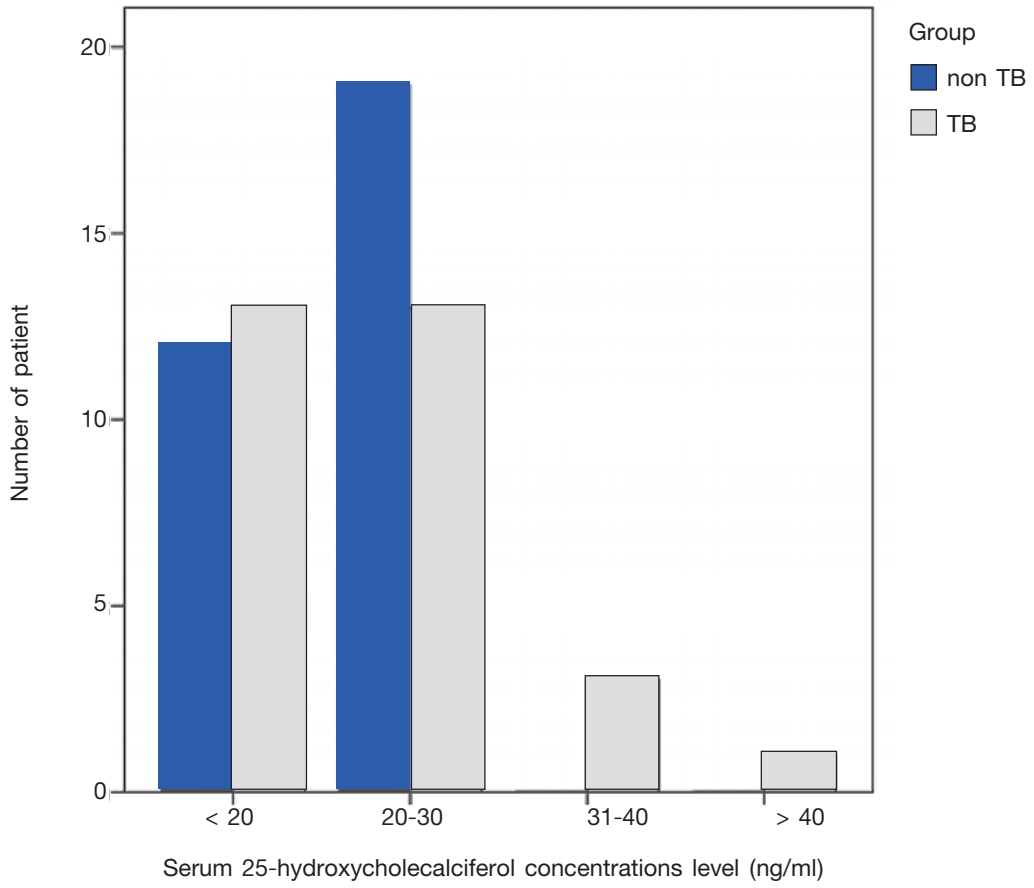


***Figure 1*** *The Level of vitamin D in TB group and control group*

## Serum 25-hydroxycholecalciferol concentrations

In TB group, mean and median of serum 25-hydroxycholecalciferol concentrations were 22.17 and 20.32 (range 10.81-40.59) ng/mL, respectively. Thirteen patients with TB had severe vitamin D deficiency whose concentration was below 20 ng/mL. There was only 1 tuberculosis patient who had adequate serum concentration of the vitamin.

In healthy control group, mean and median of serum 25-hydroxycholecalciferol concentrations were 20.74 and 20.81 (range 10.79-28.24) ng/mL. Twelve patients had severe vitamin D deficiency, and none of the healthy control group had adequate serum concentration.

We observed serum 25-hydroxycholecalciferol below 40 ng per milliliter in 90% (27 from 30 patients) of the TB patients and 100% of the healthy control group. There was no statistical significant difference between these two groups.

The proportions of various degrees of vitamin D deficiency are displayed in Figure 1.

## Serum calcium and albumin

In patients with TB, the serum calcium levels were corrected for albumin. We found that 18 patients had normal calcium level, four patients had high calcium level (more than 10.5 mg/dL) and one had low calcium level. The range of corrected calcium was 7.78 to 14.90 mg/dL. Mean calcium level was 9.9 mg/dL. High calcium level was not statistically significant correlated with TB patients.

## Discussion

In our study, we showed that vitamin D levels (25-hydroxycholecalciferol) were low in most patients with pulmonary tuberculosis (96%, 29/30), and all patients in control group (100%, 31/31). There was no difference between mean serum 25-hydroxycholecalciferol in pulmonary TB and control group ($P$ = 0.34)

Interestingly, Thailand is located in the tropical warm climate which has suggested that the level of serum 25-hydroxycholecalciferol should be normal or high. However, both groups in our study had vitamin D deficiency, except one in TB group who had normal vitamin D level. However there are many factor influencing hypovitaminosis D that we did not record such as time and duration of exposure to sunlight, dietary intake or history of use of sunblock.

As compared with previous studies, the number of patients with pulmonary tuberculosis who had vitamin D deficiency in this study was higher. In one report, prevalence of vitamin D deficiency was 76%[10] but in our study it was as high as 96%. The other difference is that the previous studies showed the evidence that vitamin D deficiency was associated with a higher risk of active tuberculosis[9,11], but our study did not demonstrate such association. The low level of serum 25-hydroxycholecalciferol in our study may be due to older mean age, especially in control group compared to previous study which was 59 and 42 years of average[14]. As we already know that vitamin D status decreases with age, mainly as a result of restricted sunlight exposure, reduced capacity of the skin to produce vitamin D, and reduced dietary vitamin D intake[15]. More than a half of our tuberculosis group and control group are unemployed, therefore they may not be exposed to sunlight, which is an important factor for vitamin D synthesis, possibly being the cause of vitamin D deficiency in our patients.

There are several limitations of our study. Firstly, we collected the small size in each group. Secondly, we did not record the other factors that influence vitamin D deficiency such as genetic determinants, environmental conditions that were associated with inadequate cutaneous synthesis; we enrolled only patients and control group in Ramathibodi Hospital, which is located in the center of Thailand. Therefore, it cannot be a good representation of overall population of Thailand.

Pulmonary tuberculosis may have vitamin D deficiency from many attributive factors including genetic determinants, inadequate synthesis due to environmental conditions medically or socially imposed isolation, and reduced dietary intake due to preference or TB-induced anorexia.

In conclusion, the serum vitamin D level is found to have no difference between pulmonary tuberculosis patients and normal population in Thailand.

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Original Articles/นิพนธ์ต้นฉบับ

# ความสัมพันธ์ระหว่างระดับวิตะมินดี และการเกิดวัณโรคปอดในประเทศไทย

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## บทคัดย่อ

**บทนำและวัตถุประสงค์:** ประมาณ 10 ล้านคนในโลกมีภาวะพร่องวิตะมินดี ซึ่งวิตะมินดีมีความสำคัญในการควบคุมการทำงานของระดับภูมิคุ้มกันในร่างกาย มีรายงานในอดีตพบความสัมพันธ์ระหว่าง polymorphysim ของตัวรับวิตะมินดีกับการเกิดวัณโรคในประชากรบางกลุ่ม และยังพบความสัมพันธ์ระหว่างภาวะพร่องวิตะมินดีกับการติดเชื้อวัณโรคในปอด ซึ่งยังไม่มีการศึกษาความสัมพันธ์ดังกล่าวในประเทศไทย

**วิธีการศึกษา:** ทำการวัดระดับ 25-hydroxycholcalcifeol แคลเซียม ฟอสเฟต ระดับครีอะตินีน การทำงานของตับ และข้อมูลทางประชากรในคนที่มีประวัติทางคลินิกและผลทางห้องปฏิบัติการที่บ่งชี้ว่าจะเป็นวัณโรคปอดที่อยู่ในระยะแพร่เชื้อ เปรียบเทียบกับกลุ่มประชากรทั่วไปที่มีอายุ-เพศที่ลักษณะคล้ายคลึงกัน แต่ไม่ได้มีอาการบ่งชี้ว่าเป็นวัณโรคปอดและมีภาพถ่ายรังสีปอดปกติ ผู้ป่วยที่มีโรคไตเรื้อรัง กลุ่มอาการเนฟโฟรติก ผู้ป่วยที่เป็นโรคตับแข็ง และผู้ป่วยที่ได้รับยาต้านไวรัสเอดส์ ยากลุ่มเสตียรอยด์ ยากันชัก และสารอาหารเสริม จะถูกคัดออกจากการศึกษาเนื่องจากจะมีผลต่อระดับวิตะมินดีในเลือด

**ผลลัพธ์:** มีผู้ป่วยวัณโรคปอด 30 ราย และกลุ่มประชากรที่เป็นกลุ่มควบคุมจำนวน 31 ราย พบว่าร้อยละ 90 (27 ใน 30 ราย) ของผู้ป่วยวัณโรคปอด และร้อยละ 100 ของกลุ่มประชากรที่เป็นกลุ่มควบคุม มีระดับวิตะมินดีต่ำกว่า 40 นาโนกรัมต่อมิลลิลิตร ซึ่งไม่มีความแตกต่างกันอย่างมีนัยสำคัญทางสถิติ

**สรุป:** ไม่พบความแตกต่างอย่างมีนัยสำคัญทางสถิติของค่า 25-hydroxycholcalciferol ในเลือดของผู้ป่วยที่วินิจฉัยวัณโรคปอดเมื่อเทียบกับประชากรกลุ่มควบคุม

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