

Levels of 1,25(OH)₂D₃ for patients with pulmonary tuberculosis and correlations of 1,25(OH)₂D₃ with the clinical features of TB

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Background: To determine the 1,25-dihydroxyvitamin D₃ [1,25(OH)₂D₃] concentrations to patients with tuberculosis (TB) and whether it influenced the patient's clinical features.

Methods: For the first part, a total of 153 healthy adults and 74 patients with pulmonary TB (PTB) were enrolled. Serum concentrations of 1,25(OH)₂D₃ were determined by liquid chromatography-tandem mass spectroscopy to examine the 1,25(OH)₂D₃ concentrations of the two groups from the peripheral blood. If there are differences between the two groups, what follow will increase the experimental group numbers to examine the relationship among the 1,25(OH)₂D₃ concentrations with the numbers of the lesion area, the tubercule bacilli in sputum and the CD4/CD8 ratio of T lymphocytes in the peripheral blood.

Results: In the first part, the 1,25(OH)₂D₃ concentrations was lower in patients with TB than in those healthy adults [365.9 (SD 235.7) *vs.* 464.3 (SD 335.6), *P*<0.05]. In the second part, we increased the sample size to 134 (male 91 cases, female 43 cases). we found that the plasma levels of 1,25(OH)₂D₃ are not correlated with the numbers of the lesion area and the tubercule bacilli in sputum, but the 1,25(OH)₂D₃ levels can interact the ratio of CD4/CD8 T lymphocytes, it shows a positive correlation with the ratio of CD4/CD8 T lymphocytes.

Conclusions: The 1,25(OH)₂D₃ concentrations in TB patients lower than the healthy adults, it might exist as a risk factor during the development of TB or TB might affect the levels of 1,25(OH)₂D₃. But the different status vitamin D concentration might not affect the numbers of the lesion area, the tubercule bacilli in sputum. It shows a positive correlation with the ratio of CD4/CD8 T lymphocytes. The study will have a significance value to clinical medicine, but further study will need to study the levels of 1,25(OH)₂D₃ with the TB.

Keywords: Tuberculosis (TB); 1,25-dihydroxyvitamin D₃ [1,25(OH)₂D₃]; clinical features; CD4/CD8 T lymphocytes

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Introduction

Vitamin D has a plethora of functions in the human body, of which the regulation of calcium metabolism is the most well-known. In recent years, the important role for vitamin D in the modulation of the innate immune by its role is as an immunomodulator (1-4). Although the effects of vitamin D on the innate immune response have been established *in vitro* and *ex vivo* (5,6), some data are available

evaluating plasma 25(OH)D concentrations (7-12), there is a few concern and studying of its active metabolite 1,25-dihydroxyvitamin D₃ [1,25(OH)₂D₃] *in vivo*. Plasma concentrations of 1,25(OH)₂D₃, but not 25(OH)D₃, is the most active form of vitamin D and is responsible for most of its biological actions, directly up-regulates the production of human cathelicidin antimicrobial peptide (CAMP) from monocytes/macrophages infected with

mycobacterium tuberculosis (TB) (13-15). 1,25(OH)₂D₃ status had a strong influence by seasonal variation (16). In addition, there are litter studies regarding 1,25(OH)₂D₃ with clinical outcomes, in this study, we have examined the 1,25(OH)₂D₃ concentration of the two groups from the peripheral blood and the relationship about the 1,25(OH)₂D₃ status among the lesion areas (the number of lesion area was divided into six areas through the lower edge of the 2,4 ribs during the chest X-rays), the tubercule bacilli in sputum. Previous *in vitro* and animal studies have suggested that 1,25(OH)₂D₃ has important immunoregulatory properties, and it has been well documented that T lymphocytes are dominant leukocytes present in TB, and the different immunophenotype of lymphocytes affect the prognosis of TB. Previous *in vitro* and animal studies have suggested that 1,25(OH)₂D₃ has important immunoregulatory properties and it has been well documented that T lymphocytes are dominant leukocytes present in TB. CD4(+) helper and CD8(+) cytotoxic T cells, two major subsets of T lymphocytes, CD4 T cells become activated and proliferate rapidly secreting cytokines that send signals and maintain active immune response. On the other hand, the CD8 T-cells destroy virally infected cells and tumor cells. The ratio of CD4/CD8 T lymphocyte cell affects the resistance in mycobacterium TB. Previous *in vitro* and animal studies have suggested that 1,25(OH)₂D₃ has important immunoregulatory properties. So, at the last, we investigated the ratio of CD4/CD8 lymphocyte cell in TB patients, to investigate whether the status of 1,25(OH)₂D₃ affects the ratio of CD4/CD8 T lymphocyte cell.

Patients and methods

Patients and data acquisition

The patients were recruited from the clinics of Nanjing Chest Hospital Tuberculosis Research Centre (TRC), who were clinically and radiologically diagnosed with PTB, which was confirmed by sputum smear examinations, culture for *M. tuberculosis* (at least two sputum specimens positive or culture for acid-fast bacilli by microscopy sputum smear positive) or clinical features. The test group and control group are come from the Eastern China. All the patients were HIV negative and none of them presented with any other infectious disease or any other organ function impaired. To this end, ethylenediamine tetraacetic acid (EDTA) anticoagulated blood was collected before

administration of medicine and centrifuged immediately at 2,000 rpm for 10 min at 4 °C, after which plasma was stored at -80 °C until analysis. Serum concentrations of 1,25(OH)₂D₃ and the ratio of CD4/CD8 T lymphocyte cell were determined by liquid chromatography-tandem mass spectroscopy, the 24 hours smear were collected to be used for concentration smear and routine culture. The number of lesions and smear-negative TB (≥ 3 sputum smear investigations is negative) or smear-positive TB (≥ 3 sputum smear investigations is positive) were obtained from the clinical history of the patients to examine the concentrations of the two groups. The lesion areas, the sputum conversion rate of the PTB were obtained from the clinical history to examine the correlations between the 1,25(OH)₂D₃ concentrations and these relations. The study was approved by the Institutional Ethics Committee of Nanjing Chest Hospital Tuberculosis Research Centre. Informed consent was obtained from each subject before the start of the study.

Statistical analyses

All analyses were performed using SPSS18.0. Student's *t*-test was applied to calculate the 1,25(OH)₂D₃ concentrations between the male and female, the sputum negative and positive, the ratio of CD4/CD8 T lymphocyte cell $\geq$ and ≤ 1.0 , one way analysis of variance (ANOVA) was used to compare the 1,25(OH)₂D₃ concentrations among the numbers of the lesion areas of two groups. P value less than 0.05 was considered statistically significant.

Results

1,25(OH)₂D₃ concentrations in the two groups

A total of 74 TB and 153 healthy adults blood were obtained, in the cases group, 46 males and 28 females, in the control group, 91 males and 72 females, among the two groups, there is no significant difference of basal clinical conditions between the two groups (Table 1). 1,25(OH)₂D₃ concentrations were found to be significantly decreased among PTB patients compared to the healthy adults [365.9 (SD 235.7) vs. 464.3 (SD 335.6), $P < 0.05$] (Figure 1). In the male group, the 1,25(OH)₂D₃ concentrations were found to be decreased among PTB patients compared to the healthy adults ($P < 0.05$), unfortunately, in the female group, there is no significant difference between the two groups ($P > 0.05$) (Table 2).

Table 1 Baseline characteristics of the two groups		
	Cases	Controls
Male	46	81
Female	28	72
Body mass index (SD)	19.1 (3.2)	19.5 (3.7)
Total number	74	153

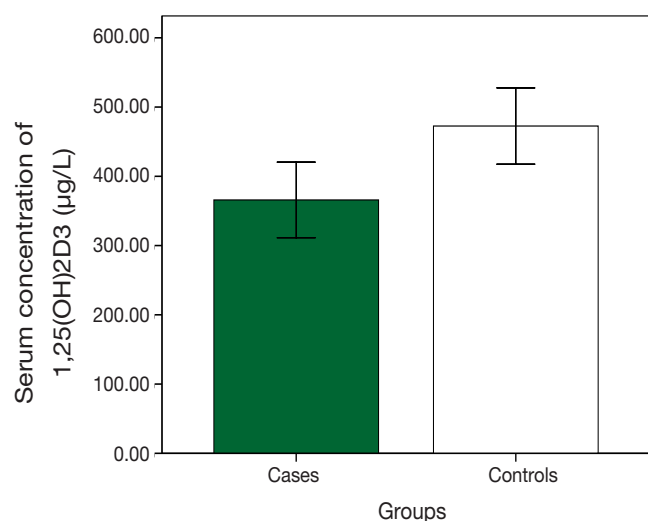


Figure 1 The plasma concentrations of 1,25(OH)2D3 of the two groups. Green stands for cases group, white stands for control group, the plasma concentrations of 1,25(OH)2D3 of the control group was greater compare with the test group. 1,25(OH)2D3, 1,25-dihydroxyvitamin D3.

Table 2 Ages and the plasma concentrations of 1,25(OH)2D3 of the two groups					
	Cases		Controls		P
	Mean	SD	Mean	SD	
Age (years)	31.2	10.4	33.07	10.6	0.193
1,25(OH)2D3 (µg/L)					
Total	365.9	235.7	464.3	335.6	0.010
Male	340.7	175.3	429.1	315.8	0.037
Female	407.1	508.7	310.2	356.2	0.188
1,25(OH)2D3, 1,25-dihydroxyvitamin D3.					

Correlation between plasma concentrations of 1,25(OH)2D3 and the numbers of the lesion area, the smear-negative or positive and the ratio of the ratio of CD4/CD8 T lymphocyte cell

In the second part, we increase the number of TB cases

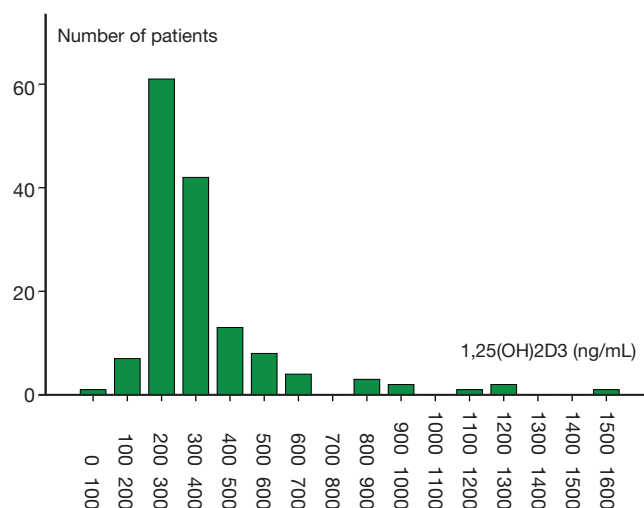


Figure 2 The number of patients of different levels of 1,25(OH)2D3 in the TB group. 1,25(OH)2D3, 1,25-dihydroxyvitamin D3; TB, tuberculosis.

to 134, males 91, females 43, the most common status 1,25(OH)2D3 is 200-600 ng/mL (Figure 2). It showed that the status of 1,25(OH)2D3 was not correlation with the different numbers of the lesion areas, the smear-negative or positive, but it shows a positive correlation with the ratio of CD4/CD8 T lymphocytes with the status of 1,25(OH)2D3 [331.0 (SD 49.7) vs. 394.8 (SD 235.7), $P < 0.05$] (Table 3).

Discussion

In the present study, vitamin D status was evaluated in view of its immune-regulatory role in susceptibility to microbial infection (17). Lots of researches have shown that 1,25(OH)2D3 can increase the monocytes/macrophages to killing mycobacterium TB (18,19). We have shown that the patients with TB had comparatively prevalence of concentrations decreased compared with those healthy adults (365.9 vs. 464.3, $P = 0.010$), supported the evidence that the TB patients reflects their poor immune status compared to healthy adults (20,21). Suggest the need for vitamin D supplementation in such patients to prevent or to adjuvant the antibiotic therapy for control of TB. There has been much hope that vitamin D might fulfill at least some of these actions as a potential adjunctive treatment in active or latent TB. As see in the Figure 2, the concentrations of 200-600 µg/L were take the most percentage both in the TB groups. In the cases group the male patients shows the

Table 3 The basic clinical and the relationship among plasma concentrations of 1,25(OH)2D3 and the numbers of the lesion areas, the smear-negative or positive and the ratio of CD4/CD8 T lymphocyte cell

Group	n	Mean	SD	P
Total	134	368.4	216.1	0.228
Male	91	354.8	184.5	
Female	43	400.9	267.3	
Smear (-) or (+)				0.105
(+)	56	301.9	96.0	
(-)	70	406.5	261.4	
The ratio of CD4/CD8				0.008
(I) ≤ 1.0	70	331.0	49.7	
(II) ≥ 1.0	64	394.8	235.7	
The numbers of lesion area				
(I) 1-2	47	343.9	193.4	(I) and (II) P=0.107
(II) 2-4	32	394.6	238.4	(II) and (III) P=0.192
(III) 4-6	55	387.2	217.5	(I) and (III) P=0.547
1,25(OH)2D3, 1,25-dihydroxyvitamin D3.				

1,25(OH)2D3 concentrations decreased than those healthy male adults (340.7 *vs.* 429.1, $P < 0.05$), but in the female group, there is no significant difference between the two groups ($P > 0.05$) (Table 2). These findings question the role of estrogen levels maybe affect the 1,25(OH)2D3 status and the active metabolite 1,25(OH)2D3 *in vivo* easy to be influenced by the environment (22). We also studied the different concentrations of 1,25(OH)2D3 on the numbers of the lesion areas and the smear-negative or positive of TB patients. It revealed that there were no significant difference between the concentrations of 1,25(OH)2D3 with the different lung fields. In the last part, we found that the 1,25(OH)2D3 levels can interact the ratio of CD4/CD8 T lymphocytes, it shows a positive correlation with the ratio of CD4/CD8 T lymphocytes, this findings also coincide with the results of Torres's (23). This result also broad our mind that maybe the 1,25(OH)2D3 can improve the immune system of TB patient.

Several limitations of our study deserve attention. First, we studied only the activate concentrations of 1,25(OH)2D3 in the two groups, and have not studied the 25(OH)D3, However, to our knowledge, there are litter data that indicate the concentrations of 1,25(OH)2D3 and

its effects on the imaging feature of TB. Secondly, we have only studied the different status of 1,25(OH)2D3 that can affect the ratio of CD4/CD8 T lymphocytes, the higher status of 1,25(OH)2D3, the higher ratio of CD4/CD8 T lymphocytes, this result will broaden our mind that the 1,25(OH)2D3 affects the ratio of CD4/CD8 T lymphocytes subtypes and let the person proved susceptible to TB. Further study will need to study the 1,25(OH)2D3 with the immune system.

Conclusions

In conclusion, the main finding of our study is that the 1,25(OH)2D3 concentrations were lower in patients with TB than in those healthy adults. And the 1,25(OH)2D3 levels can interact the ratio of CD4/CD8 T lymphocytes, it shows a positive correlation with the ratio of CD4/CD8 T lymphocytes. These data provide further evidence that lower of 1,25(OH)2D3 status may predispose to TB or the 1,25(OH)2D3 concentrations might exist as a risk factor during the development of TB, however, it is impossible to investigate the causal relationship between vitamin D3 and susceptibility for TB in this paper. Questions about the relation between TB and 1,25(OH)2D3 concentrations are largely unexplored, it can be concluded that vitamin D supplementation is a much needed, low cost, effective, and safe intervention strategy for breast cancer prevention that should be implemented.

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