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ORIGINAL ARTICLE

The Correlation between 25-Hydroxyvitamin D3 and the Severity and Short-Term Prognosis of Pulmonary Tuberculosis

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SUMMARY

Background: **Pulmonary tuberculosis is a chronic infectious disease caused by mycobacterium tuberculosis in the lungs. The present study aims to investigate the correlation between serum 25-hydroxyvitamin D3 (25-VD3) and the severity and short-term prognosis of tuberculosis.**

Methods: **The clinical data of 261 pulmonary tuberculosis patients, who were admitted to the Tuberculosis Diagnosis and Treatment Center of our hospital from January 1, 2017 to December 31, 2020, was retrospectively collected. Taking the median of 25-VD3 at admission (11.40 ng/mL) as the cutoff value, these patients were divided into two groups: high 25-VD3 group (> 11.40 ng/mL, n = 131) and low 25-VD3 group (≤ 11.40 ng/mL, n = 130). Then, Pearson's correlation analysis was performed using SPSS to determine the correlation between the 25-VD3 level and the length of hospitalization and Acute Physiology and Chronic Health Evaluation II (APACHE II) score of pulmonary tuberculosis patients. According to the clinical outcome after 28 days of treatment, these patients were divided again into two groups: improvement group (n = 170) and death group (n = 91). Then, Pearson's correlation analysis through SPSS was performed to determine the relationship between the 25-VD3 level and short-term prognosis of pulmonary tuberculosis patients.**

Results: **Compared to the low 25-VD3 group, pulmonary tuberculosis patients in the high 25-VD3 group were younger, had a higher percentage of improvement after 28 days of treatment, and had a lower APACHE II score ($p < 0.05$). However, the 25-VD3 level was not significantly correlated to the length of hospital stay of pulmonary tuberculosis patients (correlation coefficient $r = 0.020$, $p = 0.746$) and was significantly negatively correlated to the APACHE II score (correlation coefficient $r = -0.211$, $p = 0.001$). In addition, the age and APACHE II score of patients were lower in the improvement group, when compared to the death group, while the 25-VD3 level was higher and the length of hospital stay was longer, when compared to the death group ($p < 0.01$). The logistic regression analysis revealed that the length of hospital stay, APACHE II score, and 25-VD3 level are independent risk factors that affect the prognosis of pulmonary tuberculosis patients ($p < 0.05$).**

Conclusions: **In summary, 25-VD3 is closely correlated to the severity of tuberculosis, and this can be used to evaluate and predict the prognosis of patients.**

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KEY WORDS

serum vitamin D3, tuberculosis, APACHEII score, short-term prognosis, correlation study

INTRODUCTION

Background

Pulmonary tuberculosis (PTB) is a chronic infectious disease caused by Mycobacterium tuberculosis (MTB) infection in the lungs and is one of the major global public health issues [1]. China is one of the countries with a high incidence and mortality for PTB. Furthermore, the 2019 Global Tuberculosis Report issued by the World Health Organization (WHO) revealed that that there were approximately 866,000 new cases of tuberculosis and 37,000 deaths in China in 2018 [2]. Therefore, identifying an indicator that can easily be detected has a high priority rate and is closely correlated to the condition and prognosis of PTB is of great significance for the prevention and treatment of PTB in China.

In recent years, studies have shown that vitamin D (VD) is involved in the pathogenesis of MTB infection and PTB [3,4]. VD is a fat-soluble vitamin, and its active metabolite, 1,25-dihydroxyvitamin D3 (1,25 (OH)2 VD3) has the activity of inhibiting the inflammatory response involved in inflammatory cytokines [5]. The 1,25 (OH)2 VD3 binds to the vitamin D receptor (VDR) and subsequently binds to the retinoic acid X receptor to form a heterodimer, which can activate the response ability of immune cells, thereby inhibiting the growth of MTB [6]. At the same time, the combination of 1,25 (OH)2 VD3 and VDR also increases the level of human endogenous antimicrobial peptide LL-37 and enhances the inactivation of MTB through macrophages [7]. A study has proven that VD deficiency is common in PTB patients, and the development of VD deficiency is more rapid after the infection with MTB [8]. Another study revealed that the VD status of patients with sepsis syndrome in the intensive care unit is correlated to the severity of the disease [9]. However, research on the level of VD3 in active PTB and its correlation with the severity of PTB and short-term prognosis is relatively scarce. The present study retrospectively analyzed the clinical data of 261 patients in our Tuberculosis Diagnosis and Treatment Center and explored the relationship between the level of 25-VD3 and the severity and short-term prognosis of patients with PTB, aiming to provide reference for the clinical prevention and treatment of PTB.

MATERIALS AND METHODS

Participants

In the present study, the clinical data of tuberculosis patients, who were admitted to the Tuberculosis Diagnosis and Treatment Center of our hospital from January 1, 2017 to December 31, 2020, were retrospectively collected. All patients were diagnosed in accordance with the "WHO Comprehensive Guidelines: Tuberculosis-Rapid Diagnosis of Tuberculosis Test" diagnostic criteria [10]. Exclusion criteria: (1) PTB patients with negative sputum MTB culture or extrapulmonary tuberculosis patients; (2) patients with diseases that would affect the 25-VD3 level; (3) patients who used corticosteroids, diuretics, or VD supplements in the past 30 days; (4) patients presently using cytotoxic or immunosuppressive drugs; (5) patients with autoimmune diseases and HIV infection; (6) patients with incomplete clinical data. Finally, a total of 261 patients were included for the present study. Taking the median of the 25-VD3 level at admission as the cutoff value, these patients were divided into two groups: high 25-VD3 group (> 11.40 ng/mL, n = 131) and low 25-VD3 group (≤ 11.40 ng/mL, n = 130). Then, according to the clinical outcome after 28 days of treatment, these patients were divided again into two groups: improvement group (n = 170) and death group (n = 91).

PTB diagnosis

According to the Chinese Medical Association "Guidelines for the Primary Diagnosis and Treatment of Tuberculosis (2018)", PTB was diagnosed based on the following: (1) Epidemiological characteristics: close contact with infectious PTB. (2) Clinical symptoms: cough and sputum for three weeks or more, which may be accompanied by chest pain, dyspnea, low afternoon fever, hemoptysis, night sweats, fatigue, loss of appetite, weight loss, menstrual disorders, erythema nodosum, vesicular conjunctivitis, and tuberculosis rheumatism. (3) Imaging performance: The posterior apical segment of the upper lung lobe, dorsal segment of the lower lung lobe, and posterior basal segment have manifestations, such as exudation, proliferation, fibrosis, and caseous lesions, or the presence of bronchial dissemination, calcification, pleural effusion, pleural thickening and adhesions. These may be surrounded by satellite lesions, and there may be bronchial drainage signs on the medial end, which can easily merge with the cavities, and may present as miliary shadows in the lungs. (4) Etiological basis: The sputum MTB isolation and culture were positive.

25-VD3 test

The retrospective study was conducted by accessing the electronic medical records and collecting the following clinical data of the study subjects: gender, age, 25-VD3 level, final outcome (improvement/death), length of hospital stay, Acute Physiology and Chronic Health Evaluation (APACHE) score, etc. The 25-VD3 level was determined from the peripheral venous blood collected on an empty stomach in the morning of the next day after admission.

Statistical analysis

The measurement data was expressed as mean ± standard deviation ($\overline{x} \pm s$), while the count data usage rate was expressed in percentage (%). The SPSS 20.0 statistical software was used to perform the *t*-test for the measurement data, and the X^2-test for the count data. Pearson's correlation analysis was used to determine the

Table 1. Comparison of baseline data between the low 25-VD3 group and high 25-VD3 group.

Group	n	Gender (male/female)	Ages (years)	Final outcome (improvement/death)	Length of hospital stay (days)	APACHE II score
Low 25-VD3 group	131	93/39	57.5 ± 24.0	68/63	28 (13,54)	23.27 ± 9.77
High 25-VD3 group	130	100/30	67.4 ± 16.9	102/28	26.5 (14,45)	20.75 ± 8.01
t or X^2		1.503	-3.852	20.258	-0.164	2.276
p		0.220	0.000	0.000	0.870	0.024

Table 2. Comparison of data between the improvement group and death group.

Group	n	Gender (male/female)	Age (years)	25-VD3 (ng/mL)	Length of hospital stay (days)	APACHE II score
Improvement group	170	120/50	59.86 ± 21.64	14.37 ± 8.34	32.5 (18.75, 51.00)	17.89 ± 5.92
Death group	91	73/19	67.25 ± 19.89	9.89 ± 5.64	14.00 (6.00, 31.00)	29.71 ± 8.87
t or X^2		2.219	-2.703	4.595	-6.068	-12.850
p		0.136	0.007	0.000	0.000	0.000

Table 3. The logistic regression for factors that affect the prognosis of PTB patients.

Index	B	SE	Wald X^2	p	OR	OR (95% CI)
Number of days in the hospital	-0.023	0.007	9.866	0.002	0.978	0.964 ~ 0.992
APACHE II score	0.188	0.029	42.817	0.000	1.207	1.141 ~ 1.277
VD3	-0.127	0.034	14.208	0.000	0.881	0.824 ~ 0.941
Age	0.017	0.010	2.936	0.087	1.017	0.998 ~ 1.036
Gender (female vs. male)	0.591	0.424	1.948	0.163	1.806	0.787 ~ 4.143
Constant	-4.175	0.943	19.582	0.000	0.015	-

correlation between the 25-VD3 level and length of hospital stay of PTB patients and their APACHE II score. Binary logistic regression analysis was used to determine the factors that affect the prognosis of PTB patients after 28 days of treatment. $p < 0.05$ was considered statistically significant.

RESULTS

Comparison of baseline data between the high 25-VD3 group and low 25-VD3 group

The results for the comparison of baseline data are presented in Table 1. There was no statistical difference in gender ratio and length of hospital stay between the low 25-VD3 group and high 25-VD3 group ($p > 0.05$). Furthermore, patients were older in the high 25-VD3 group when compared to the low 25-VD3 group ($p < 0.001$). In the final outcome, the improvement was greater when compared to the low 25-VD3 group ($p < 0.001$), while the APACHE II score decreased in the low VD3 group ($p = 0.024$).

The correlation analysis for the 25-VD3 level and length of hospital stay of patients with PTB

Pearson's correlation analysis results revealed that there was no significant correlation between the serum 25-VD3 level and length of hospital stay of PTB patients (correlation coefficient $r = 0.020$, $p = 0.746$).

The correlation analysis between the 25-VD3 level and APACHE II score of PTB patients

Pearson's correlation analysis result is presented in Figure 2. The serum 25-VD3 level of PTB patients was significantly negatively correlated with the APACHE II score (correlation coefficient $r = -0.211$, $p = 0.001$).

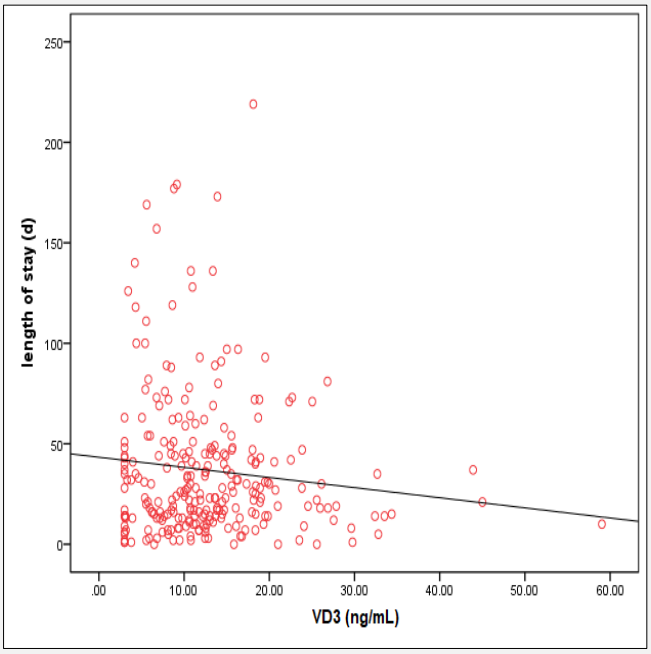

Figure 1. The correlation analysis between the serum VD3 levels and length of hospital stay of pulmonary tuberculosis patients.

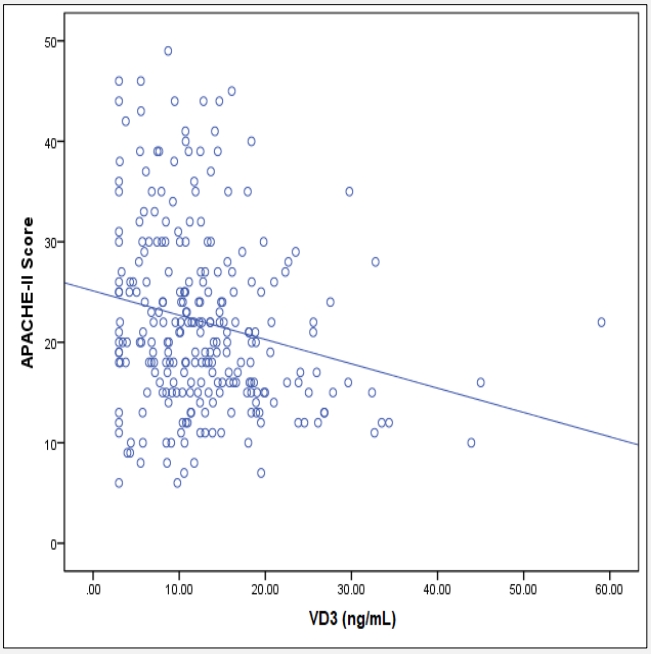

Figure 2. The correlation analysis between the serum VD3 level and APACHE II score of pulmonary tuberculosis patients.

Comparison of data between the improvement group and death group

The results for the comparison of baseline data are presented in Table 2. There was no statistical difference in gender ratio between the improvement group and death group ($p > 0.05$). Furthermore, the age ($p = 0.007$) and APACHE II score were lower in the improvement group, when compared to the death group ($p < 0.001$). Moreover, the 25-VD3 level was higher and the length of hospital stay was longer when compared to the death group ($p < 0.001$).

The logistic regression for factors that affect the prognosis of PTB patients

The binary logistic regression analysis results for factors that affect the prognosis of PTB patients are presented in Table 3. The length of hospital stays, APACHE II score, and 25-VD3 level were the independent risk factors that affected the prognosis of PTB patients ($p < 0.05$). However, age and gender had no significant effect on the prognosis of PTB patients ($p > 0.05$).

DISCUSSION

After MTB invades the human body, the body's immune system stops the disease from progressing by controlling its replication. Patients merely have a positive MTB test with no clinical manifestations. However, when the body's immunity is reduced, MTB will restart its replication, rapidly progress to active PTB, and trigger the corresponding clinical symptoms, transforming into a new source of infection [11]. MTB infection and the onset of PTB are closely correlated to the immune status of the body. These two form a process of increasing and decreasing each other [12]. However, these above conclusions are only partial reports. Furthermore, there are still many questions and debates on the role of PTB immune factor levels and the incidence of tuberculosis infection and its mechanism. In particular, the relationship between serum VD and the PTB condition and prognosis remains unclear.

VD is a lipid-soluble endosterol derivative that can regulate the expression of a variety of genes in cells, including genes correlated to the maintenance of calcium homeostasis and bone metabolism, lymphocyte-released cytokine genes, and a variety of genes correlated to the proliferation of vascular smooth muscle cells. In addition, its role in innate immunity and acquired immunity has received attention. The results of two studies from different groups indicated that VD is closely correlated to the progression of PTB, and that the frequency of PTB attacks is closely correlated to the lack of VD [13, 14]. The 25-VD3 is the active metabolite of VD. In kidney cells, 25-OH-D3-1-hydroxylase catalyzes the production of 1,25-dihydroxyvitamin D3 [1,25(OH)2 VD3, 1,25-VD3]. Previous *in vitro* experiments have confirmed that 1,25-VD3 can regulate the immune response and enhance the ability of cells to kill MTB. Furthermore, 1,25-VD3 can bind to toll-like receptors on macrophages, promote the expression of antimicrobial peptides and 1α-hydroxylase, and enhance the ability of macrophages to kill MTB [15]. Salamon et al. reported that 1,25-VD3 can inhibit the formation of lipid droplets in MTB-infected macrophages and the biological activity of peroxisome proliferator-activated receptor γ in cells [16]. In turn, this inhibits the growth of MTB in cells and improves the ability of macrophages to kill MTB. In addition, 1,25-VD3 can also promote autophagy in monocytes, and enhance the body's ability to fight MTB by directly killing bacteria and regulating cytokines.

Due to the low content of active VD in blood and its short half-life, the plasma concentration would become unstable. At present, 25-VD3 is internationally used to reflect the level of active VD in blood. The study conducted by Wang et al. revealed that the levels of 25-VD3 and 1,25-VD3 were significantly lower in PTB patients, when compared to healthy people (51.60 ± 27.25 nmol/L vs. 117.50 ± 75.50 nmol/L, $p < 0.001$; 82.63 ± 51.43 pmol/L vs. 94.02 ± 49.26 pmol /L, $p = 0.03$) [17]. Furthermore, they found that there was an interaction between the methylation level of the key gene CYP-27A1 in the VD metabolic pathway and the 25-VD3 concentration and that both are correlated to PTB risk (OR = 4.11, 95% CI = 1.26 - 13.36, $p = 0.019$). Moreover, the serum 25-VD3 level at the end of the intensive treatment was also correlated to the patient's prognosis ($p = 0.008$). Majewski K et al. reported that the serum 25-VD3 level of patients with active PTB was significantly lower than that of healthy people, and that the serum 25-VD3 level of patients with severe PTB was significantly lower than that of patients with mild PTB [18]. This indicates that the serum 25-VD3 level of patients with active PTB is correlated to the severity of PTB lesions. Hence, when the VD in the body is insufficient, its restriction on MTB and the regulation of immune function of the body are also significantly reduced, thereby affecting the occurrence and severity of PTB.

Based on the above literature reports, the present study retrospectively collected the clinical data of 261 patients with PTB. Taking the median of 25-VD3 at admission (11.40 ng/mL) as the cutoff value, it was found that patients with PTB in the high 25-VD3 group were relatively young. Furthermore, the proportion of improved patients was higher, and the APACHE II scores were lower, Hence, there is a significant negative correlation between the serum 25-VD3 level and APACHE II score of PTB patients. At the same time, these PTB patients were further grouped according to their clinical outcome. It was found that the age and APACHE II score were lower, the level of 25-VD3 was higher, and the length of hospital stay was longer for patients in the improvement group, when compared to the death group ($p < 0.01$). Furthermore, the regression analysis for factors that affect the prognosis of PTB patients revealed that the length of hospital stay, APACHE II score, and 25-

VD3 level are the independent risk factors that affect the prognosis of PTB patients ($p < 0.05$). In summary, the level of 25-VD3 is closely correlated to the severity and prognosis of PTB patients, and this can be used to evaluate and predict the prognosis of patients. The investigators also speculate that high serum 25-VD3 levels may help improve the condition and prognosis of patients with PTB. In future studies, the investigators will further determine which dose of VD supplementation can help improve the final outcome of PTB patients, as well as the influencing factors for the serum 25-VD3 level of PTB patients, in order to further improve the study.

Declaration of Interest:
None.

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