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Serum vitamin D level and vitamin D receptor genotypes may be associated with tuberculosis clinical characteristics

A case–control study

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Abstract

Vitamin D is associated with the susceptibility of tuberculosis and might have an adjunctive effect on anti-tuberculosis treatment. This study aims to investigate the association of vitamin D deficiency and vitamin D receptor (VDR) gene polymorphisms with susceptibility and severity to multidrug-resistant tuberculosis (MDR-TB) in comparison with drug-sensitive tuberculosis (DS-TB) and health controls in China.

A total of 180 patients with pulmonary TB (128 DS-TB, 52 MDR-TB) and 59 healthy controls were enrolled into 3 groups. Vitamin D levels and VDR genotypes at *FokI, BsmI*, *ApaI*, and *TaqI* sites of all the participants and clinical characteristics of patients with TB were measured and collected.

Statistical analysis revealed that vitamin D levels were lower in both TB groups. Patients with TB with bilateral lesions and patients with MDR-TB with extrapulmonary TB had lower vitamin D levels. The frequencies of ff genotype and f allele were higher in both TB groups. Patients with Ff genotype and ff genotype had lower proportion of extrapulmonary TB. Patients with ff genotype had higher proportion of retreatment. Male patients with ff genotype had higher proportion of cavity formation. Patients with DS-TB with AA genotype had higher proportion of cavity formation.

Our findings demonstrate that vitamin D deficiency and ff genotype may be the risk factors of TB in Chinese population. In addition, patients with TB with lower level of vitamin D may have a greater risk of bilateral lesions and extrapulmonary TB. VDR genotypes may be associated with TB clinical characteristics.

Abbreviations: DS-TB = drug-sensitive tuberculosis, MDR-TB = multidrug-resistant tuberculosis, MTB = *Mycobacterium tuberculosis*, TB = tuberculosis, VDR = vitamin D receptor.

Keywords: clinical characteristics, tuberculosis, vitamin D deficiency, vitamin D receptor gene polymorphisms

1. Introduction

Tuberculosis (TB), which is caused by *Mycobacterium tuberculosis* (MTB), is still an on-going and predominant health problem in the world. World Health Organization (WHO) estimated that there were 10.4 million people developed the disease with 1.7 million succumbing to it worldwide in 2016.[1] The number of TB deaths is unacceptably high and it appears to be a serious challenge to control the rise for a relatively long period of time due to the increase morbidity of drug-resistant TB and co-epidemic of TB and HIV.[2]

High doses of vitamin D were widely used to treat TB in the preantibiotic era,[3,4] and the active metabolite of vitamin D, 1, 25-dihydoxyvitamin D, has long been known to enhance the immune response to mycobacteria in vitro.[5] In recent years, there is an increasing number of studies focusing on exploring the relationship between vitamin D deficiency and TB.[6,7] However, existing attempts to relate these 2 things have been made with conflicting results which may be caused by the different study populations. Here, we studied the association between vitamin D and TB in Chinese population. We measured vitamin D levels and vitamin D receptor (VDR) genotypes at *FokI*, *BsmI*, *ApaI*, and *TaqI* sites of all the participants, collected clinical characteristics of patients with TB. Combination with all these data, we

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Table 1

Specific primer sequences for determination of vitamin D receptor gene genotypes.

	Forward primer	Reverse primer
FokI	5′-AGCTGGCCCTGGCACTGACTCTGCTCT-3′	5′-ATGGAAACACCTTGCTTCTTCTCCCTC-3′
BsmI	5′-ACCAAGACTACAAGTACCGCGTCAGTG- 3′	5′-AACCAGCGGGAAGAGGTCAAGGG-3′
ApaI	5′-CAGAGCATGGACAGGGAGC-3′	5′-AGGAGAGGCAGCGGTACTG-3′
TaqI	5′-CAGAGCATGGACAGGGAGC-3′	5′-AGGAGAGGCAGCGGTACTG-3′

investigated the association from different angles in drug-resistant and drug-sensitive patients using healthy individuals as controls.

To our knowledge, no studies have tried to investigate the clinical characteristics with vitamin D deficiency and VDR genotypes in Chinese patients with TB. This study sets out to assess the vitamin D level and the prevalence of VDR single-nucleotide polymorphisms of all participants, and primary clinical characteristics in patients, and to explore the links among them in different groups.

2. Materials and methods

2.1. Patient recruitment

This study was conducted at Beijing Chest Hospital, which is the only national referral TB center in China. A total of 180 active pulmonary patients with TB and 59 healthy volunteers were recruited between February 2015 and February 2016. All patients were diagnosed by specialist physicians. Patients who failed to diagnose active pulmonary TB, who have taken vitamin D recently, or who refused to complete the whole test, were excluded. Patients with drug susceptibility testing revealing MDR tuberculosis strains were enrolled into multidrug-resistant tuberculosis (MDR-TB) group. Patients with drug-sensitive MTB were enrolled into drug-sensitive tuberculosis (DS-TB) group. The healthy volunteers were participants without a history of TB or TB exposure, without significant respiratory symptoms. Participants who have a history of cancer or HIV positive were also excluded. This study plan had been approved by our hospital medical ethics committee and all the participants signed informed consent.

2.2. Sample collection and processing

Blood samples were obtained under fasting conditions. About 2 mL of venous blood samples were collected from study participants in ethylenediaminetetraacetic-acid-containing tubes and centrifuged 15 minutes at 3000 rpm. Serum was separated and transferred to new tubes and stored at −80°C until the vitamin D detection, and the rest of the anticoagulation was used for DNA extraction.

2.3. Measurement of vitamin D levels

Vitamin D level was defined as the serum concentration of 25 (OH)D, combination of 25(OH)D2 and 25(OH)D3 concentrations, which is generally accepted as the best evaluation indicator for vitamin D status due to its stability and long half-life. Serum concentrations of 25(OH)D were measured in 180 patients and 59 healthy controls by an established ultra-high performance liquid chromatography–tandem mass spectrometry procedure which has been published in 2016.[8] It was performed on a 4000 QTRAP liquid chromatography with mass spectrometry (LC-MS/MS) system, which is consisted of Eksigent ekspert Ultra LC 110 liquid chromatograph (AB Sciex, Framingham, MA) and an AB Sciex 4000 Q TRAP triple-quadrupole mass spectrometer (AB Sciex, Framingham, MA).

2.4. Genotyping for VDR polymorphisms

The genomic DNA of the participants was extracted from peripheral blood leukocytes using a DNA blood genomic DNA isolation kit (TIANGEN DP318, Beijing, China) according to the manufacturer's protocol. The concentration and purity of the DNA samples were checked by BioSpectrometer ultraviolet spectrophotometer (Eppendorf Basic 22331, Hamburg, Germany). The detection of the polymorphisms, including *FokI*, *BsmI*, *ApaI*, and *TaqI* of the VDR gene was carried out using the conventional polymerase chain reaction (PCR)-restriction fragment length polymorphism method by a thermocycler (BIO-RAD S1000, Beijing, China) for PCR amplification. The primer sequences are provided in Table 1, while the reaction conditions are provided in Table 2. The PCR products and restriction enzyme products were electrophoresed in a 1% to 2% agarose gel and then stained with nucleic acid dye (SBS Good View, Beijing, China). The presence of a restriction site was denoted by lower case (f, b, a and t, for *FokI*, *BsmI*, *ApaI*, and *TaqI*) and absence by upper case (F, B, A, and T, for *FokI*, *BsmI*, *ApaI*, and *TaqI*). This method was similar to that previously reported[9] and we verified

Table 2

Reaction conditions for determination of vitamin D receptor gene genotypes.

PCR cycle	*FokI* site		*BsmI* site		*ApaI* site		*TaqI* site	
Predenaturation	94°C	10 min	94°C	3 min	94°C	5 min	94°C	5 min
Denaturation	94°C	30 s	94°C	20 s	94°C	30 s	94°C	30 s
Annealing	60°C	30 s	62°C	40 s	68°C	30 s	68°C	30 s
Extension	72°C	30 s	72°C	1 min	72°C	30 s	72°C	30 s
Insulation	72°C	10 min	72°C	10 min	72°C	10 min	72°C	10 min
Store	4°C	∞	4°C	∞	4°C	∞	4°C	∞

PCR = polymerase chain reaction.

Table 3

Clinical and demographic characteristics of the study groups.

	DS-TB (n = 128)	MDR-TB (n = 52)	Healthy controls (n = 59)	P*
Age, y	38.03 ± 16.37	40.02 ± 13.38	49.25 ± 5.35	
Sex ratio, male:female	98:30	35:17	43:16	
BMI, kg/m^2	21.26 ± 3.65	21.45 ± 3.66	24.74 ± 3.18	<.001†
Retreatment			NA	
Yes	15 (11.7%)	34 (65.4%)		
No	113 (88.3%)	18 (34.6%)		<.001
Sputum smear test			NA	
Positive	50 (39.1%)	40 (76.9%)		
Negative	78 (60.9%)	12 (23.1%)		<.001
Radiographic severity			NA	
Unilateral	35 (27.3%)	10 (19.2%)		
Bilateral	93 (72.7%)	42 (80.8%)		.255
Radiographic cavity			NA	
Yes	17 (13.3%)	17 (32.7%)		
No	95 (74.2%)	19 (36.5%)		
Multiple	16 (12.5%)	16 (30.8%)		<.001
Diabetes mellitus			NA	
Yes	21 (16.4%)	7 (13.5%)		
No	107 (83.6%)	45 (86.5%)		.621
Extrapulmonary TB			NA	
Yes	35 (27.3%)	16 (30.8%)		
No	93 (72.7%)	36 (69.2%)		.644

BMI = body mass index, DS-TB = drug-sensitive tuberculosis, MDR-TB = multidrug-resistant tuberculosis.

* $P < .05$ was considered statistically significant.

† BMI of patients with DS-TB and MDR-TB was compared with healthy controls, both P values were <.001.

our results by gene sequencing. The PCR sequencing technology service in this study was provided by Beijing Ruibio BioTech Co., Ltd, Beijing, China.

2.5. Collection and processing of data

We collected the demographic information and the detection results of vitamin D levels and the VDR polymorphisms. We also collected the clinical characteristics, including drug-resistant situation, sputum smear test results, radiographic severity, and radiographic cavity, whether they were retreatment patients, and whether they had diabetes mellitus or extrapulmonary TB. Clinical information of patients is kept and provided by the medical record department of our hospital. Setting 12 ng/mL (30 nmol/L) and 20 ng/mL (50 nmol/L) of 25(OH)D concentrations as the cut-off points,[10,11] we divided the concentrations of vitamin D into 3 levels, including deficiency, inadequate, and adequate. According to the drug susceptibility testing results, all the patients with TB were divided into DS-TB and MDR-TB.

Unless any special instructions, all results are presented as mean (standard deviation) or case number (proportion). The Student t test was used to test for the differences between serum 25(OH)D concentrations of patients and the controls, between serum 25(OH)D concentrations of patients with different clinical characteristics. The Chi-squared or Fisher exact test was used to test for differences in the genotypes and allele distributions of the *FokI*, *BsmI*, *ApaI*, and *TaqI* polymorphisms between patients with TB and healthy controls, between patients with different clinical characteristics. Odds ratio (OR) and 95% confidence interval (CI) were calculated to evaluate the relative disease risks according to the different genotypes and allele distributions. The significance level was .05. SPSS program (SPSS 19.0 version for Windows, Chicago) was used for all statistical analyses.

3. Results

A comparison of the demographic information and clinical characteristics of DS-TB (n = 128), MDR-TB (n = 52), and healthy controls (n = 59) is shown in Table 3. Both 2 group patients had a significantly lower BMI ($P < .001$) than the healthy controls, but there was no statistical difference between the 2 groups of patients with TB. As expected, the patients with MDR-TB had a significantly higher proportion of retreatment patients ($P < .001$), higher proportion of sputum smear-positive ($P < .001$), and higher proportion of cavity formation ($P < .001$).

3.1. Relationship between the level of vitamin D and TB

The detailed data on comparison of the concentrations of 25 (OH)D of all the participants were provided in Table 4. Significantly lower mean 25(OH)D concentrations were found in patients with TB ($C = 10.62$ ng/mL) than in the healthy controls ($C = 21.97$ ng/mL) according to the Student t test result ($P < .001$).

Based on the relevant information to further investigate the effects of demographic and clinical characteristics on vitamin D level, we found that the 25(OH)D concentrations of DS-TB ($C = 10.42$ ng/mL) and MDR-TB ($C = 11.11$ ng/mL) were both significantly ($P < .001$) lower than healthy controls, without statistically significant difference between the 2 TB groups ($P = .336$). In accordance with the standards of the WHO, setting 45 and 60 as the cut-off points, we divided patients into 3 groups by age: youth, middle age, and old age, while no significant difference was found after comparing the serum mean concentrations of 25(OH)D in different age ranges ($P = .269$). In patients with TB, patients with unilateral lesions ($C = 11.98$ ng/mL) had higher vitamin D level ($P = .030$) than those with bilateral lesions ($C = 10.17$ ng/mL). In MDR-TB, participants not combined with

Table 4

Vitamin D status of the study groups.

	n	25(OH)D concentration, ng/mL	Deficiency (<12 ng/mL)	Inadequate (12≤20 ng/mL)	Adequate (≥20 ng/mL)	P*
Healthy controls	59	21.97±6.90	4 (6.8%)	21 (35.6%)	34 (57.6%)	
Patients	180	10.62±4.86	127 (70.6%)	44 (24.4%)	9 (5.0%)	
DS-TB	128	10.42±5.06	93 (72.7%)	29 (22.7%)	6 (4.7%)	<.001
MDR-TB	52	11.11±4.35	34 (65.4%)	15 (28.8%)	3 (5.8%)	<.001
Sex ratio						
Male	133	10.77±4.83	94 (70.7%)	32 (24.1%)	7 (5.3%)	
Female	47	10.19±4.97	33 (70.2%)	12 (25.5%)	2 (4.3%)	.492
Age groups, y						
Youth (<45)	115	10.35±4.95	85 (73.9%)	24 (20.9%)	6 (5.2%)	
Middle age (45–60)	51	11.02±4.41	34 (66.7%)	15 (29.4%)	2 (3.9%)	
Old age (>60)	14	11.37±5.79	8 (57.1%)	5 (35.7%)	1 (7.1%)	.269
Drug resistance						
Yes	52	11.11±4.35	34 (65.4%)	15 (28.8%)	3 (5.8%)	
No	128	10.42±5.06	93 (72.7%)	29 (22.7%)	6 (4.7%)	.395
Retreatment						
Yes	49	11.08±4.76	32 (65.3%)	13 (26.5%)	4 (8.2%)	
No	131	10.45±4.91	95 (72.5%)	31 (23.7%)	5 (3.8%)	.438
Radiographic severity						
Unilateral	45	11.98±5.76	29 (64.4%)	12 (26.7%)	4 (8.9%)	
Bilateral	135	10.17±4.46	98 (72.6%)	32 (23.7%)	5 (3.7%)	.030
Radiographic cavity						
Yes	34	10.01±4.12	26 (76.5%)	7 (20.6%)	1 (2.9%)	
No	114	10.62±5.17	80 (70.2%)	28 (24.6%)	6 (5.3%)	
Multiple	32	11.27±4.50	21 (65.6%)	9 (28.1%)	2 (6.3%)	.577
Diabetes mellitus						
Yes	28	10.81±4.48	21 (75%)	7 (25%)	0 (0%)	
No	152	10.59±4.49	106 (69.7%)	37 (24.3%)	9 (5.9%)	.827
Extrapulmonary TB†						
Yes	16	8.89±2.05	15 (93.8%)	1 (6.3%)	0 (0%)	
No	36	12.09±4.74	19 (52.8%)	14 (38.9%)	3 (8.3%)	.001

DS-TB = drug-sensitive tuberculosis, MDR-TB = multidrug-resistant tuberculosis.

* $P < .05$ was considered statistically significant.

† The comparison was made among patients with MDR-TB.

extrapulmonary TB ($C = 8.89$ ng/mL) had higher vitamin D levels ($P = .001$) compared to those combined with extrapulmonary TB ($C = 12.09$ ng/mL).

3.2. Relationship between the VDR gene polymorphisms and TB

Genotypic and allelic frequencies and distributions of *FokI*, *BsmI*, *ApaI*, and *TaqI* polymorphisms were provided in Table 5. *FokI* site had a higher mutation frequency, especially in the patients, while the mutation frequencies of the other sites were relatively lower. Frequencies of ff genotype in DS-TB and MDR-TB were 41.4% and 50.0%, respectively. Frequencies of f allele in DS-TB and MDR-TB were 65.2% and 68.3%, respectively. These 2 groups of patients had relatively higher frequencies of ff genotype and f allele, with statistical significance ($P < .001$). The ff genotype was found to be significantly associated with DS-TB (OR 6.12, 95% CI 1.61–8.32) and MDR-TB (OR 6.00, 95% CI 2.03–17.74). We also found the f allele was associated with both DS-TB (OR 2.47, 95%CI 1.58–3.85) and MDR-TB (OR 2.83, 95% CI 1.63–4.90) significantly. There were no statistically significant differences in the genotypic and allelic distributions of the other sites.

With the clinical data, we found significant differences in the genotypic distributions of *FokI* ($P < .001$) polymorphisms between the 2 groups with or without extrapulmonary TB (Table 6). With further investigation, we found that patients with Ff genotype (OR 0.16, 95% CI 0.06–0.45) and ff genotype (OR 0.35, 95%CI 0.13–0.93) had lower proportion of extrapulmonary TB (Table S1, http://links.lww.com/MD/C370). In addition, patients with ff genotype (OR 2.09, 95% CI 1.08–4.07) had higher proportion of retreatment (Table S2, http://links.lww.com/MD/C370), male patients with ff genotype (OR 2.17, 95% CI 1.06–4.44) had higher proportion of cavity formation (Table S3, http://links.lww.com/MD/C370), and patients with DS-TB with AA genotype (OR 3.69, 95% CI 1.12–12.18) had higher proportion of cavity formation (Table S4, http://links.lww.com/MD/C370).

3.3. Relationship between the level of vitamin D and the VDR gene polymorphisms

There was a significant difference in the levels of vitamin D between the patients of different genotype groups at *BsmI* site ($P = .026$). Patients with bb genotype ($C = 10.94$ ng/mL) had a higher vitamin D level. The other sites did not show any link between the vitamin D level and the gene polymorphisms. Detailed data are provided in Table 7.

4. Discussion

Vitamin D has regained the attention of researchers devoting to TB in recent years. Based on the previous studies, researchers have made some noteworthy progress, but no studies have

Table 5

Vitamin D receptor gene polymorphisms of the study groups.

		Healthy controls (n = 59)	DS-TB (n = 128)	OR (95% CI)	P*	MDR-TB (n = 52))	OR (95% CI)	P*
FokI								
Genotype frequency	FF	21 (35.6%)	14 (10.9%)	1		7 (13.5%)	1	
	Ff	25 (42.4%)	61 (47.7%)	3.66 (1.61–8.32)	.001	19 (36.5%)	2.28 (0.80–6.47)	.117
	Ff	13 (22.0%)	53 (41.4%)	6.12 (1.61–8.32)	<.001	26 (50.0%)	6.00 (2.03–17.74)	<.001
Allele frequency	F	67 (56.8%)	89 (34.8%)	1		33 (31.7%)	1	
	F	51 (43.2%)	167 (65.2%)	2.47 (1.58–3.85)	<.001	71 (68.3%)	2.83 (1.63–4.90)	<.001
BsmI								
Genotype frequency	bb	54 (91.5%)	111 (86.7%)	1		48 (92.3%)	1	
	Bb	4 (6.8%)	15 (11.7%)	1.82 (0.58–5.76))	.300	4 (7.7%)	1.13 (0.27–4.75)	1
	BB	1 (1.7%)	2 (1.6%)	0.97 (0.09–10.97)	1	0 (0%)	0.98 (0.95–1.02)	1
Allele frequency	b	112 (94.9%)	237 (92.6%)	1		100 (96.2%)	1	
	B	6 (5.1%)	19 (7.4%)	1.50 (0.58–3.85)	.400	4 (3.8%)	0.75 (0.21–2.72)	.753
ApaI								
Genotype frequency	aa	36 (61.0%)	64 (50.0%)	1		30 (57.7%)	1	
	Aa	21 (35.6%)	50 (39.1%)	1.34 (0.70–2.57)	.380	17 (32.7%)	0.97 (0.44–2.17)	.944
	AA	2 (3.4%)	14 (10.9%)	3.94 (0.85–18.31)	.063	5 (9.6%)	3.00 (0.54–16.59)	.249
Allele frequency	a	93 (78.8%)	178 (69.5%)	1			1	
	A	25 (21.2%)	78 (30.5%)	1.63 (0.97–2.73)	.062	27 (26.0%)	1.30 (0.70–2.43)	.402
TaqI								
Genotype frequency	TT	52 (88.1%)	113 (88.3%)	1		47 (90.4%)	1	
	Tt	7 (11.9%)	14 (10.9%)	0.92 (0.35–2.42)	.866	5 (9.6%)	0.79 (0.24–2.66)	.703
	tt	0 (0%)	1 (0.8%)	1.01 (0.99–1.03)	1	0 (0%)	NA	NA
Allele frequency	T	111 (94.1%)	240 (93.8%)	1		99 (95.2%)	1	
	t	7 (5.9%)	16 (6.3%)	1.06 (0.42–2.64)	.905	5 (4.8%)	0.80 (0.25–2.60)	.712

All comparisons were made to healthy controls.

CI = confidence interval, DS-TB = drug-sensitive tuberculosis, MDR-TB = multidrug-resistant tuberculosis, OR = odds ratio.

* $P < .05$ was considered statistically significant.

investigated the clinical characteristics with vitamin D deficiency and VDR gene genotypes in Chinese patients with TB. Many studies have described the characteristics of study patients with TB, some have even suggested that low level of vitamin D may result to the relapse of TB and might have an adverse effect to the clinical improvement,[12] but there were no studies that focused on the relationship between the clinical characteristics of TB and the level of vitamin D or VDR polymorphisms at *ApaI* site, except a few studies that have talked about the relations at *FokI*, *BsmI*, and *TaqI* sites. In addition, as the concentrations of the vitamin D level were always measured by HPLC, which may not have been entirely accurate, we developed an ultra-performance LC-MS/MS method to determine the concentrations of 25(OH)D in serum.

There are increasing evidences to support the significant role of vitamin D in adjusting the immune response to TB as an immunomodulator,[13] following several lines of studies, including basic and clinical researches and a number of observational studies.[14–16] Our result which has showed patients with TB have significant lower levels of vitamin D was accord with the most of results reported in literatures.[17,18] In addition, we found that patients with bilateral lesions and extrapulmonary TB had lower vitamin D levels, which suggest vitamin D deficiency might be a risk factor of extensive lesions.

Vitamin D exerts its cellular functions through the vitamin D receptor, which is a ligand-activated transcription factor in the nucleus.[19] Four well-known polymorphisms (*FokI*, *BsmI*, *ApaI*, and *TaqI*) of VDR gene were reported to alter the immune response and be associated with susceptibility to MTB. Although several surveys have been performed in the past and reported that genetic variants might play an important role in TB susceptibility in different individuals, they had shown inconsistent and conflicting results,[20] and a few people attempted to investigate the relationship with clinical characteristics of patients with TB. In this study, we grouped the participants according to the levels of vitamin D and contrasted the mean levels and proportions of different levels. We also divided the patients into different groups according to the clinical characteristics and VDR genotypes. We found that the frequencies of ff genotype and f allele were higher in DS-TB and MDR-TB compared with healthy controls, which is consistent with a study in India,[21] and it has not been reported

Table 6

Vitamin D receptor gene polymorphisms and clinical characteristics of tuberculosis (TB) group.

	FokI - *P* value	*BsmI* - *P* value	*ApaI* - *P* value	*TaqI* - *P* value
Drug resistance	.396	.628	.643	1.000
Retreatment	.294	.605	.262	.707
Cavity formation	.288	.828	.080	.419
Diabetes mellitus	.395	.349	.812	.312
Extrapulmonary TB	<.001	.282	.684	.486

* $P < .05$ was considered statistically significant.

Table 7

Vitamin D status of different vitamin D receptor genotype of patients with tuberculosis.

Genotype frequency	25(OH)D concentration, ng/mL	P^*	Genotype frequency	25(OH)D concentration, ng/mL	P^*
FokI		.234	*ApaI*		.596
FF	11.04±4.51		AA	11.52±5.88	
Ff	9.93±3.96		Aa	10.26±4.66	
ff	11.21±5.68		aa	10.70±4.81	
BsmI		.026	*TaqI*		.316
BB	4.28±1.47		TT	10.79±5.00	
Bb	8.63±3.08		Tt	9.07±3.38	
bb	10.94±4.96		tt	12.68	

* $P < .05$ was considered statistically significant.

that f allele maybe a risk factor of TB in Chinese population. We also first found that patients with Ff genotype and ff genotype had lower proportion of extrapulmonary TB. Patients with ff genotype had higher proportion of retreatment, male patients with ff genotype had higher proportion of cavity formation. These were not found in the present study. Considering the higher proportion of retreatment and cavity in patients, ff genotype may not only be the risk factor of TB susceptibility, but also have an adverse effect on the progress and prognosis of TB. All these results suggested that the *FokI* VDR polymorphisms may be associated with the susceptibility and some clinical characteristics of active TB. Besides that our study analyzed the relationship between *ApaI* site polymorphism and patient clinical features, which has not been reported before, and found that AA genotype maybe also a risk factor of cavity formation. These observations in different VDR genotypes might imply their underlying important role in susceptibility and severity of TB.

As we all know, MDR-TB is more difficult to cure and has a higher mortality rate. A lot of researches have focused on the association between MDR-TB and vitamin D. *BsmI* polymorphism in VDR has been linked to the clinical outcomes in India, a study reported bb genotype is associated with an increased risk of smear positive and MDR-TB,[22] while another cross-sectional study found that Bb genotype has a lower risk for developing MDR-TB.[21] Besides, t allele is also considered to be correlated positively with MDR-TB.[21] However, no studies have investigated these associations in Chinese population. We collected the drug resistance situation and added it to analyses, we demonstrated that MDR-TB has a significantly lower vitamin D level than healthy control, but it shows no difference with DS-TB. And we found that ff genotype maybe a risk factor of MDR-TB, which is consistent with the previous study in Indians.[21] Rathored et al found that Ff genotype and t allele were correlated positively with MDR-TB and Bb genotype was correlated inversely with MDR-TB in Indians,[21] but our data do not show more association at these sites.

In this study, we found that the frequencies of VDR genotypes in Chinese population were significantly different from other races. The proportions of BB genotype, AA genotype, and tt genotype were 1.7%, 3.4%, and 0%, respectively, in the control group, while the data were 25%, 51.8%, and 14%, respectively, in India[21] and 18.6%, 37.1%, and 14.3%, respectively, in Turkey.[23] These lower gene mutation frequencies make the sample sizes relatively small and increase the difficulty to find some possible correlations, and this problem is also the main limitation of this study. Another limitation was that the daily diet and outdoor activity time of the participants were different, which would have a certain effect on the concentration of 25(OH)D. Additionally, the blood samples were collected in the course of treatment, the concentration of 25(OH)D might also be affected by anti-tuberculosis drugs.

Vitamin D has known important physiologic function in humans, it is essential for regulation of calcium and phosphate metabolism, and it plays a significant role in many infectious diseases, including tuberculosis. The low serum level of vitamin D may result to the relapse of TB and it would have an adverse effect to the clinical improvement.[12] In turn, adequate vitamin D could accelerate the conversion of sputum smear and culture,[24] even the patients with MDR-TB.[21] As vitamin D seems to have a great positive supplementary role, our observation and investigation made in this study may can pave the path for further trials of vitamin D supplementation in novel anti-tubercular drug therapy.

In conclusion, our study shows that vitamin D deficiency and ff genotype may also be the risk factors of TB in Chinese population. And we have some main findings: patients with TB with lower level of vitamin D may have a greater risk of bilateral lesions and extrapulmonary TB, and VDR gene polymorphisms might be associated with clinical characteristics of patients with TB.

Author contributions

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