International Journal of Advanced Biotechnology and Research (IJBR)
ISSN 0976-2612, Online ISSN 2278–599X,
Vol-8, Issue-3, 2017, pp2140-2148
http://www.bipublication.com

Research Article

VDR Gene Polymorphisms in Susceptibility to Pulmonary Tuberculosis among the LUR Population of Lorestan Province of Iran

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ABSTRACT
The vitamin D receptor (VDR) mediates the immunological function of vitamin D3, which activates macrophages has been associated to tuberculosis risk. The aim of this study was to determine whether vitamin D receptor (VDR) FokI, TaqI, BsmI, and ApaI polymorphisms are associated with the susceptibility to pulmonary tuberculosis (TB) in the Lur population of Iran.
This case-control study included 100 patients with pulmonary tuberculosis age and sex matched and 100 healthy controls in a Lur population living in Lorestan province. Polymorphisms of VDR were analyzed by using polymerase chain reaction-restriction fragment length (PCR-RFLP) technique. Association analyses were performed with the SPSS 21 statistical software.
The distribution of BsmI (Bb) genotype polymorphism were significantly higher frequency in TB patients compared to controls (61% vs. 45%, P=0.0336, OR=1.912, %95 CI=1.089–3.356). However, the allelic and genotypic frequencies of FokI, TaqI and ApaI polymorphisms were not significantly different between the patients and the controls.
Our findings demonstrated that BsmI (Bb) polymorphism may increase the susceptibility to pulmonary TB in the Lur population of Iran. We suggest that genotypes of many polymorphic genes are associated with TB, it is necessary to further more studies with larger sample size and explore the mechanism of genotypes in susceptibility to tuberculosis.

Keywords: VDR; Pulmonary Tuberculosis; Lur population

INTRODUCTION

Pulmonary tuberculosis (PTB), which is caused by the acid fast bacillus, Mycobacterium tuberculosis (MTB), is a major cause of morbidity and mortality in human populations worldwide (1). Each year more than 9 million novel cases are infected by PTB and more than 1.7 million succumb to PTB annually (2). Tuberculosis (TB) is responsible for an estimated 1.5 million deaths in 2014 (3). Approximately 10% of patients who are infected with MTB are known to progress to clinical disease (4,5). Almost one third of the world population is infected by MTB.

Additionally, the course and duration of disease vary in different individuals (6). This suggests that individual differences may act upon the susceptibility to tuberculosis and that contact with this microorganism does not always result in infection. These differences may be due to host factors and genetic sensitivity of different individuals to this disease (7,8). The identification of factors which increase disease susceptibility has potential to inform control strategies.

Susceptibility to PTB is influenced by host genetic background (9). One of which is KIR3DS1 gene and it combines with HLA-B Bw4 and Ile80 ligand (10,11). Also, other studies showed Toll like Receptors (TLRs) (12-16), Mannose-Binding Lectin (MBL) (17), Interlukin-10 (IL-10) (18) and Tumor Necrosis Factor (TNF) (19), has roles in the susceptibility or resistance to TB. Polymorphisms in vitamin D receptor (VDR) genes have been found to be associated with TB in different ethnic groups.

Vitamin D plays a role in human innate immunity to infectious agents including MTB (20). The activity of vitamin D is dependent on the vitamin D receptor (VDR). Case control studies have previously reported independent associations between vitamin D deficiency and susceptibility to active TB (21). The active form of vitamin D, is an important factor that modulates the activity of different defense and immune cells, including lymphocytes, monocytes, macrophages and epithelial cells (22,23).

The VDR gene is located on chromosome 12q13.11 (24), contains 11 exons and spans approximately 75 kb of genomic DNA (25). Three polymorphisms, BsmI (rs1544410) and ApaI (rs7975232), both in intron 8, and TaqI (rs731236) in exon 9, have been identified at the 3'-end of the gene (26). FokI (rs2228570) polymorphism is located at the VDR start codon (24). Vitamin D is involved in interleukin-2 inhibition, antibody production, and lymphocyte proliferation (27). 1,25-dihydroxy vitamin D3 inhibits interferon secretion and negatively regulates IL-12 production by down-regulating nuclear factor-kappa B (28). During a tuberculosis infection, vitamin D binds to VDR in macrophages, and activates synthesis of cathelicidin, which restricts MTB intracellular growth in macrophages (29,30), and eliminates MTB in phagolysosomes (31). Many studies showed an association between vitamin D deficiency and diseases such as systemic lupus erythematosus (32), diabetes mellitus (33), tuberculosis (34), etc. So, VDR polymorphisms may contribute to pulmonary tuberculosis susceptibility.

Previous studies have not reached a consensus regarding the association between the VDR gene polymorphisms and the susceptibility to PTB. In Gambian (23) and London population, (35) a significant interaction between FokI and TaqI and susceptibility to PTB was observed, while another study in Cambodians (36) not found association between the VDR gene and PTB. Many studies have examined the potential contribution made by VDR polymorphisms to PTB susceptibility, but the findings have been contradictory (35-47).

Due to the differences between distribution of VDR gene in different races and nations and also due to association of VDR polymorphisms with PTB susceptibility, in this study, we evaluated the prevalence of these polymorphisms in PTB patients and healthy controls and role of VDR gene polymorphisms on PTB susceptibility by conducting an extensive association analysis of a case– control study in a Lur population dwelled in Lorestan Province. Therefore, in the present study, we explored whether the VDR FokI, TaqI, BsmI, and ApaI polymorphisms are associated with the susceptibility to PTB.

MATERIAL & METHODS

Patients and controls

The study was approved from the Ethical Committee of Lorestan University of Medical Sciences (LUMS.REC.1395.113). Informed consent was taken from all participants before sample collection. All subjects agreed to take part in the study. We conducted a case control study. Cases were 100 unrelated Lur individuals referred to the health center of Khorramabad city of Lorestan Province. The inclusion criteria in the

case group were newly diagnosed pulmonary tuberculosis patients, Lur ethnic, have symptoms of PTB, positive sputum smear and chest radiography consistent with active disease. Patients with diabetes mellitus, ischemic heart disease, chronic renal failure, consuming immunosuppressive drugs, any autoimmune disease, any chronic inflammatory disease, jaundice or seropositivity for hepatitis B surface antigen, Hepatitis C Virus or Human Immunodeficiency Virus were excluded. All patients received TB standard treatment and none of them had drug resistance. Controls were 100 unrelated Iranian individuals of the same race and geographic region. Controls subjects had no history PTB and no evidence of prior PTB noted on chest radiography. The healthy control group composed of healthy individuals who matched in age and gender with the patient group. Additionally, all study subjects had parents of the same race.

Genotyping

Blood samples were collected from 100 pulmonary tuberculosis patients and 100 healthy controls. We extracted genomic DNA from peripheral blood samples using the QIAmp kit (Qiagen, Germany) according to the manufacturer's protocol. Our study was performed to determine the distribution of gene polymorphisms by using Polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP), that previously suggested by Sharma et al (48) and Selvaraj et al (49). PCR-RFLP analysis was used to identify the polymorphisms of VDR gene. For the VDR gene, the TaqI, FokI, ApaI and BsmI polymorphisms were detected in patients and controls using their genomic DNA. The list of forward and reverse primer sequences, restriction enzymes and digestion patterns for different alleles were assorted in Table 1.

Table 1-Primer sequences, restriction enzymes used and restriction digestion patterns for genotyping of VDR polymorphisms.

VDR polymorphisms	Sequences of the primers	Restriction enzymes	Length of the restriction fragments for different alleles
Fok A/G (rs2228570)	F: 5'AGCTGGCCCTGGCACTGACTCTGCTCT3'	FokI	265
	R: 5'ATGGAAACACCTTGCTTCTTCTCCCTC3'		196, 69
Taq C/G (rs731236)	F: 5'CAGAGCATGGACAGGGAGCAA3'	TaqI	2000
	R: 5'CACTTCGAGCACAAGGGGCGTTAGC3'		1800, 200
Apa C/T (rs7975232)	F: 5'CAACCAAGACTACAAGTACCGCGTCAGTGA3'	ApaI	2000
	R: 5'CACTTCGAGCACAAGGGGCGTTAGC3'		1700, 300
Bsm G/T (rs1544410)	F: 5'CAACCAAGACTACAAGTACCGCGTCAGTGA3'	BsmI	825
	R: 5'AACCAGCGGGAAGAGGTCAAGGG3'		650, 175

PCR amplifications were performed using purified DNA Mastercycler (BioRad, USA) in 20 µl reaction volumes. The polymorphisms of VDR gene were genotyped by FokI, TaqI, ApaI and BsmI. Thermocycling parameters were as follows: initial denaturation at 94^0C for 4 min, followed by 32 cycles at 94^0C for 30 s, at 65^0C for 30 s and at 72^0C for 1 min (ApaI and TaqI) (48). For BsmI initial denaturation step at 94^0C for 4 min, followed by 35 cycles at 94^0C for 30 s, annealing 63^0C for 30 s and extension 72^0C for 60 s and finally 2 min extension at 72^0C. For FokI initial denaturation at 94^0C for 4 min, followed by 35 cycles at 94^0C for 30 s, annealing 58^0C for 30 s and extension 72^0C for 1 min and finally 2 min extension at 72^0C (49). The PCR products were incubated at 37^0C for 3h with ApaI and FokI, 65^0C for 3h with TaqI and BsmI restriction enzymes, to digest the DNA. The restriction digestion products were separated by electrophoresis on a 1.5% agarose gel, stained with ethidium bromide, and finally observed

under ultraviolet light. The genotypes were defined according to generated fragment patterns. To ensure the validity of result, we also did the DNA sequencing on the 5% representative isolates to check the fragment generated from RFLP.

STATISTICAL ANALYSIS

The genotypic and allelic frequencies of FokI, TaqI, ApaI and BsmI polymorphisms were ascertained by direct counting in the PTB patients and control group. Departure from Hardy-Weinberg Equilibrium (HWE) of All polymorphisms frequency was assessed by an exact test in both patient and control groups. Statistical analyses were done with SPSS 21. We calculated differences in the genotypic and allelic frequencies of FokI, TaqI, ApaI and BsmI polymorphisms between the cases groups and control groups by Chi-square test and Fisher's exact test (50). All of the p-values presented in this study are two-sided, and $P \leq 0.05$ was used as the threshold of statistical significance. Odds ratios (OR) and 95% confidence intervals (CIs) were calculated to quantify the degree of association between the polymorphisms and tuberculosis (51).

RESULTS

A total of 100 cases with smear-positive PTB and 100 healthy controls were recruited to the study between September 2016 and November 2016. The mean age of cases was (32.43 ± 4.36, range 16-49 years) and the mean age of controls was (35.82 ± 5.18, range 18-55 years). 43 individuals of cases were males and 57 individuals were females, and 52 individuals of controls were males and 48 individuals were females.

The genotype distribution of VDR polymorphisms in all subjects did not deviate from the Hardy-Weinberg equilibrium ($P>0.05$). The results of the association between the VDR FokI (rs2228570), TaqI (rs731236), BsmI (rs1544410) and ApaI (rs7975232) genotypic and allelic frequencies and the risk of PTB were listed in Table 2 and 3.

Table 2- Distribution of VDR genotypes in TB patients group and healthy controls group.

VDR polymorphisms	Genotypes	% of TB patients group	% of Healthy controls group
VDR-FokI	FF	58	60
	Ff	38	35
	ff	4	5
VDR-TaqI	TT	51	54
	Tt	39	41
	tt	10	5
VDR-ApaI	AA	41	37
	Aa	52	50
	aa	7	13
VDR-BsmI	BB	7	17
	Bb	61*	45
	bb	32	38
* Significant difference (P<0.05), n=100.			

Table 3- Distribution of VDR alleles in TB patients group and healthy controls group

VDR polymorphisms	Alleles	% Allele frequency in TB patients group	% Allele frequency in Healthy controls group
VDR-FokI	F	77	77.5
	f	23	22.5
VDR-TaqI	T	70.5	74.5
	t	29.5	25.5
VDR-ApaI	A	67	62
	a	33	38
VDR-BsmI	B	37.5	39.5
	b	62.5	60.5
* Significant difference (P<0.05).			

The genotypic frequencies of VDR FokI, TaqI and ApaI polymorphisms did not have significant difference between the PTB and the controls. Based on the analysis of loci, we found that the BsmI polymorphism of the VDR gene were associated with the occurrence of tuberculosis. We observed the genotype "Bb" of BsmI polymorphism increased the PTB risk. For VDR BsmI (rs1544410) polymorphism, the genotype "Bb" (OR=1.912; 95% CI=1.089-3.356; P=0.0336) increased PTB risk by 1.912-fold among patients compared with controls (61% vs. 45% respectively) (Table 2).

The allelic frequencies of VDR FokI, TaqI, BsmI and ApaI polymorphisms did not have significant difference between the patients and the controls (Table 3).

DISCUSSION

Tuberculosis is a major public health threat. The susceptibility to TB is multifactorial disease, influenced by environmental and host genetic factors (52-55). The host immune response against MTB is mediated by cellular immunity (9). VDR gene may be involved in genetic susceptibility to TB. The compound $1,25(OH)_2$ D3 restricts the growth of MTB in monocytes by inducing the expression of the antimicrobial peptide cathelicidin (56). Vitamin D status involved in the activation of macrophages and restricts the intracellular development of MTB (57). This effect of vitamin D may be influenced by VDR gene polymorphisms. VDR polymorphisms might therefore result in an altered immune response. In previous studies, there is no research reported that the association between VDR gene and PTB susceptibility in the Lur population of Iran. We for the first time studied the correlations between VDR gene polymorphisms FokI (rs2228570), TaqI (rs731236), ApaI (rs7975232), BsmI (rs1544410) and PTB susceptibility in this population. The genotypic frequencies of VDR BsmI (Bb) polymorphism have significant difference between PTB patients and the healthy group. The genotypic and allelic frequencies of VDR FokI, TaqI, ApaI polymorphism showed no correlations with PTB susceptibility By contrast, our study did not replicate previous studies of associations between VDR polymorphisms and susceptibility to PTB (58,59).

The relation between VDR gene polymorphisms and susceptibility to TB has been studied in different populations. Wei et al. reported no association was found between susceptibility to PTB and VDR polymorphisms in the Chinese Han population (60). VDR gene polymorphisms have been associated with TB resistance in Gambia, and data showed a significantly lower frequency of TaqI (tt) genotype among TB patients (23). Also, a study in Cambodia (36) and Tanzania (47) found no associations between VDR gene and PTB. Our results are inconsistent with some previous studies, a possible explanation could be their ethnic differences. In Tuvinians from Tuva Republic and Russians from Tomsk city, no association between PTB and Fok I genotype was found (61).

Our results showed similar outcome as a study that carried out in PTB patients of south India. In their study, the VDR BsmI (Bb) genotype was associated with susceptibility to TB, whereas VDR ApaI (AA) genotype and BsmI (BB) genotype were associated with resistance to pulmonary tuberculosis (45,62). The similar studies also showed that FokI may be associated with TB, while TaqI polymorphism was not associated with TB development in Chinese Han and Kazak populations (23).

Merza et al. (63), Banoei et al. (39) in the Iranian population, and Ates et al. (37) in Turkish population, find statistically significant association between BsmI polymorphism and PTB. In contrast, Rashidi et al. (64), in the Iranian population, did not find statistically significant relationship between FokI, BsmI, ApaI and TaqI polymorphisms in VDR gene and susceptibility to tuberculosis. These paradoxical findings could be caused by gene-environment interaction, gene-gene interaction, and gene-agent interactions (65,66).

Our comprehensive analysis of VDR polymorphisms suggests that VDR BsmI (Bb)

genotype associate with PTB risk in the Lur population of Iran. This study will lead to a better understanding of the immunological and genetic pathways in TB. Finally, we suggest that further studies should involve other regions and ethnic groups with larger sample size. Further studies are required to determine how VDR polymorphisms influence susceptibility to Mycobacterium tuberculosis infection. These studies will be helpful for the prediction of high risk populations.

CONFILCT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

We are grateful to all of the patients and individuals for their participation. This study was supported by the Lorestan University of Medical Sciences under Grant No. 1586-1.

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