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Short Communication

Vitamin D Receptor Gene Polymorphism and Vitamin D Plasma Concentration: Correlation with Susceptibility to Tuberculosis

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Abstract

***Purpose*:** It is estimated that one third of the world's population were infected with *M. tuberculosis*, but only 10% of them have developed in to disease form. This subject refers to differences in host immune system activity against the tuberculosis. Vitamin D and its receptor (VDR) are important factors in the host innate immune system against the tuberculosis. In the present study VDR gene polymorphisms and its relationship with plasma vitamin D levels in susceptibility to tuberculosis have been investigated.

***Methods*:** The subjects were 84 patients with tuberculosis and 90 healthy controls. Vitamin D levels were measured in all study participants. DNA was isolated from the blood leukocytes of all groups and amplified by polymerase chain reaction (PCR). Then restriction fragment length polymorphism (RFLP) was performed on each PCR products to study the VDR gene polymorphisms. The statistical analyses were conducted using SPSS.

***Results*:** There was no statistically significant relationship between polymorphisms of *Fok*I, *Bsm*I, *Apa*I and *Taq*I in VDR gene and susceptibility to tuberculosis. Vitamin D deficiency and susceptibility to tuberculosis were closely related (95% CI -0.08 – 4.7, $P = 0.059$). Also the relationship between plasma vitamin D levels and frequency of *Fok*I-ff gene polymorphism was significant in all study participants ($P = 0.045$).

***Conclusion*:** When the genotype frequencies of VDR gene polymorphisms were analyzed with respect to plasma vitamin D levels, a significant association was seen. As an enhancement in plasma vitamin D levels in individuals (with *Fok*I-ff genotype and low levels of vitamin D) may protect them against active tuberculosis.

Introduction

Tuberculosis still remains an important cause of morbidity and mortality worldwide. Most of the cases were of pulmonary type, which can easily be spread to others by coughing and breathing.[1,2] In addition, the HIV infection, which weakens the immune system of the host has been an important reason for the spread of tuberculosis during the present years.[3] One in 10 infected persons develops clinical disease, and the majority of them have only latent TB infection.[4] Innate immune responses have a crucial role in determining the development of infectious disease.[5] Vitamin D and proper activity of its receptor are two important factors against tuberculosis in this system. The clinical studies have proven that the conjunction of the active form of vitamin D with human monocytes, which have the receptors for this vitamin, lead to increase in the attachment of phagosome to lysosome inside the macrophages. It also stimulates synthesis of free radical (nitric oxide: NO) which increases the ability of these cells against bacilli growth.[6,7] Active form of this vitamin is the main ligand of the vitamin D receptor on the nuclear of the macrophages.[8] Both the plasma vitamin D concentration and proper action of its receptor on the macrophages nuclear have important roles as parts of the innate immune system. These are two concepts that influence the susceptibility or resistance against tuberculosis, which can be investigated.[5,9] There are four important gene polymorphisms (*Fok*I, *Bsm*I, *Apa*I, *Taq*I) in VDR gene. They have been recognized by PCR-RFLP which influence the activity of the VDR which leads to susceptibility or resistance to tuberculosis.[6,10,11] The aim of this study is to investigate the relationship between VDR gene polymorphisms and their association with plasma vitamin D levels in susceptibility to tuberculosis.

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Materials and Methods

This study was performed on 84 patients with tuberculosis (50 males and 34 females), who had referred to the Tuberculosis and Lung Diseases Research Center in Tabriz and Health Center of Ardabil from the beginning of 2012 until the end of the same year. Tuberculosis in the case group was proven by preparation of direct smear from the sputum of the people referred, Ziehl-Neelsen staining , cultivation of them on Lowenstein-Jensen medium (Hi-Media, Bombay, India).[12] The control group, 90 people (49 males and 41 females) were selected from the healthy staff of Tuberculosis and Lung Diseases Research Center in Tabriz and Health Center of Ardabil and suspected people whose affliction was investigated and rejected by the clinical and Para clinical examinations.[12]

The genomic DNA was isolated from Buffy coat leukocytes of both groups by modified Proteinase K (Fermentas, St. Leon- Rot, Germany), Sodium dodecyl sulfate (SDS), Cetyltrimethylammonium bromide (CTAB), (E. Merck, Darmstadt, Germany) protocol.[13]

Genotyping of VDR Gene

In this study, the polymorphisms of *Fok*I, *Bsm*I, *Apa*I and *Taq*I in VDR gene were investigated. PCR was performed on genomic DNA and specific primers[14,15] (Generay Biotech, Shanghai, China) in a thermal cycler (Peq lab, primus 96, Erlangen, Germany). A 740 bp segment containing the *Taq*I and *Apa*I polymorphisms was amplified using 5'- CAG AGC ATG GAC AGG GAG CAA -3', 5'-GCA ACT CCT CAT GGC TGA GGT CTC- 3' and a 825 bp segment containing the *Bsm*I polymorphism was amplified using 5'-CAA CCA AGA CTA CAA GTA CCG CGT CAG TGA-3', 5'-AAC CAG CGG GAA GAG GTC AAG GG-3' and a 265 bp segment containing the *Fok*I polymorphism was amplified using 5'-AGC TGG CCT GGC ACT GAC TCT GCT CT-3', 5'-ATG GAA ACA CCT TGC TTC TTC TCC CTC-3' on the final concentration 0.5 µM in the presence of $MgCl_2$ (1.5 mM), each dNTP (100µM), KCl (50mM), Tris-Cl (20 mM), pH 8.4 and Taq DNA Polymerase enzyme (Cinnagene, Tehran, Iran).

DNA samples were amplified with cycling parameters as follows: Initial denaturation step at 94°C for 7 min followed by 35 cycles of denaturation at 94°C for 45 Sec, annealing at 67°C (for *Apa*I, *Taq*I), 70°C (for *Bsm*I) and 65°C (for *Fok*I) for 50 Sec and extension at 72°C for 65 Sec, and completed by 7 min at 72°C as final extension. After performing all steps of PCR, the products followed by 1% agarose gel (Cinnagene, Tehran, Iran) electrophoresis. The PCR products were digested with *Apa*I and *Bsm*I enzymes (10 U at 37°C), *Taq*I enzyme (10 U at 65°C), and *Fok*I enzyme (10 U at 55°C), (Thermo Scientific, Espoo, Finland). The final products of PCR-RFLP were electrophoresed on 2% agarose gel (for *Bsm*I, *Apa*I, *Taq*I) and 2.5% agarose gel for *Fok*I polymorphisms together with the size marker (100 bp-Plus, Thermo Scientific, Espoo, Finland).

Vitamin D estimation

Total plasma 25-hydroxyvitamin D (25-OHD) was measured for all study participants by ELISA, all protocols were followed according to manufacturer's instructions (DLD Diagnostika GMBH-Hamburg, Germany).

Statistical analysis

All reported confidence intervals (CI_s) are 95% CI_s, and all *P* values were two tailed; $P<0.05$ was considered to be statistically significant. The mean values were compared using Student's t-test for parametric variables. The Chi-square test was used to compare the frequency of variables. Multivariate analysis was performed using binary logistic regression. All statistical analyses were conducted using SPSS (version 14; SPSS).

Results

PCR products restricted with *Fok*I reveals genotypes (after treatment with enzyme) denoted FF (265 bp), Ff (265,196 and 69 bp), or ff (196 and 69 bp) and *Bsm*I genotypes denoted BB (825 bp), Bb (825, 650 and 175 bp), bb (650 and 175 bp) and *Apa*I genotypes denoted AA (740 bp), Aa (740, 530 and 210 bp), aa (530 and 210 bp) and *Taq*I genotypes denoted TT (495 and 245 bp), Tt (495, 290, 245 And 205 bp), tt (290, 245 and 205 bp). Allele and genotype frequencies of VDR gene polymorphisms in the patients and normal group were analyzed by SPSS V.14 program as shown in Table 1. All genotype frequencies of VDR gene were no significant association with 25-OHD concentration except *Fok*I-ff genotype (Table 2).

However, in the frequency of the BB genotype of *Bsm*I polymorphism and the tt genotype of *Taq*I polymorphism increased trends were observed in the patient group in contrast healthy control group. Sex and age relationship in susceptibility to tuberculosis was no statistically significant: (OR = 0.78, 95% CI 0.43 – 1.42, $P = 0.41$) and (Mean Difference = 4.1, 95% CI-10.45 – 2.2, $P = 0.2$), respectively. Plasma 25-OHD concentrations and tuberculosis were closely related: (Mean difference = 2.30, 95% CI -0.08 – 4.7, $P = 0.059$).

Discussion

Plasma vitamin D levels and the polymorphisms in VDR gene have been studied in relation to tuberculosis in many populations.[16] In population- based case-control studies, the actual roles of candidate host genes tend to be confounded by environmental and intrinsic individual factors, such as BCG immunization, smoking, alcohol drinking, nutrition status and sunlight exposure.[4,17] Considering the combinational and synergistic effects of environmental factors with genetic factors such as the combination of 1, 25-$(OH)_2D_3$ and interferon-γ (which is more effective than either agent alone) in restricting the growth of *M. tuberculosis* in monocytes,[18] we investigated the plasma 25-OHD concentrations with VDR gene polymorphisms in susceptibility to tuberculosis. The relationship between VDR gene

polymorphisms and serum 25-OHD levels are subjects that have not investigated much. There was a high prevalence of 25-OHD deficiency in Gujarati Asians (with *Taq*I-TT or Tt genotypes) who were living in London and the incidence of tuberculosis was reported to be high among them. The lowest serum 25-OHD concentrations were found in patients with active disease. Although patients with active disease showed a trend towards a lower frequency of the tt genotype, the results of the studies by Bhanushali et al [10] in Indian population showed higher levels of vitamin D in *Taq*I-tt individuals than non *Taq*I-tt.

Table 1. Genotype and allelic frequencies for single-nucleotide polymorphisms (SNPs) in the vitamin D receptor (VDR) in all patients with tuberculosis (TB) and control subjects.

Genotypes	Frequency (%)		Odds Ratios	95% CI	*P* Value
	Patients	Healthy (Control)			
***Bsm*I-BB**	27(32.14)	26(28.89)	1		
Bb	27(32.14)	31(34.44)	1.076	0.53-2.21	0.84
bb	30(35.72)	33(36.67)	0.597	0.29-1.25	0.17
Total	84	90			
Alleles					
B	81(48.22)	83(46.11)	1		
b	87(51.78)	97(53.89)	1.088	0.71-1.66	0.69
***Taq*I-TT**	35(41.67)	38(42.22)	1		
Tt	34(40.48)	41(45.56)	1.64	0.67-4.05	0.28
tt	15(17.85)	11(12.22)	1.064	0.43-2.6	0.89
Total	84	90			
Alleles					
T	104(61.9)	117(65)	1		
t	64(38.09)	63(35)	0.875	0.565-1.35	0.55
***Fok*I-FF**	44(52.4)	50(55.6)	1		
Ff	33(39.3)	32(35.5)	1.17	0.62 –2.2	0.62
ff	7(8.3)	8(8.9)	0.99	0.33 – 2.96	0.99
Total	84	90			
Alleles					
F	121(72)	132(73.3)	1		
f	47(27.7)	48(26.7)	1.15	0.67 – 1.7	0.65
***Apa*I-AA**	29(34.53)	30(33.3)	1		
Aa	42(50)	48(53.3)	0.91	0.47 – 1.7	0.77
aa	13(15.47)	12(13.4)	1.12	0.4 – 2.8	0.8
Total	84	90			
Alleles					
A	100(59.5)	108(60)	1		
a	68(40.5)	72(40)	0.98	0.64 – 1.5	0.9

CI: Confidence Interval

Table 2. Correlation between genotype frequencies of VDR gene polymorphism and 25-OHD levels in both groups.

Genotype	Mean concentration (ng/ml)	F	*P* value (ANOVA)
***Bsm*I-BB**	15.3		
Bb	16.1	0.47	0.62
bb	14.3		
***Fok*I-FF**	15.7		
Ff	14	3.04	0.045
ff	20.8		
***Taq*I-TT**	15.5		
Tt	15.8	0.46	0.64
tt	14.2		
***Apa*I-AA**	14.8		
Aa	15.7	0.38	0.69
aa	14.6		

ANOVA: analysis of variance

Our results in this study were significantly associated (P= 0.045), as individuals with normal levels of 25-OHD (≥ 20 ng/ml) and a low prevalence of tuberculosis showed a trend towards a higher frequency of the *Fok*I-ff genotype, which suggests the probability of protection against the tuberculosis. Although the frequency of alleles and genotype relationship in susceptibility to tuberculosis was analyzed alone, without respect to 25-OHD levels, a significant association was not seen.

The *Fok*I restriction site defines a SNP in the first of two potential translations – initiation start sites for VDR mRNA. Thus, two protein variants can exist; the long VDR is encoded in the alternate allele form (ATG) (designated f) has three more amino acids and is 1.7 times less efficient than the VDR encoded by the common allele form (ACG) (designated F).[11] In the present study, the higher frequency of *Fok*I-ff genotype

in individuals with high concentration of vitamin D in comparison with the people who have low levels of this vitamin may indicate a reparative state. Decreased function of VDR protein in *Fok*I individuals might be repaired with high levels of vitamin D. Although several researchers have proved the association of tuberculosis and gene polymorphisms without respect to environmental factors such as the studies which had been carried out on Romanian population,[19] there was an increased frequency of homozygote genotypes TT in controls rather than in patients. In another study on Paraguayans population, Wilbur et al.[20] showed that *Taq*I-t allele and *Fok*I-F allele protects the individual against the active form of disease and infectious form of tuberculosis, respectively. The researches which have been conducted on *Fok*I, *Bsm*I, *Apa*I and *Taq*I polymorphisms of VDR gene in the three countries of Gambia, Guinea and Guinea Bissau,[21] along with the findings of the study by CAO Shang et al.[8] in China on Han ethnic population and our results in this study did not show any significant relationship between the case and control groups in susceptibility to tuberculosis. In Iran, the other studies by Marashian et al.[22] showed that the polymorphisms of *Taq*I and *Bsm*I in VDR gene did not have any statistically difference in the genotype frequencies of case and control groups. The findings in our study indicated that vitamin D deficiency (≤ 10 ng/ml) (especially in *Fok*I-ff individuals) can carry a higher risk of tuberculosis; therefore moderate supplementation may be useful. According to the results of a clinical trial in Ahvaz city by Alavi et al.[23] on TB patients, vitamin D supplementation could accelerate the negation of the direct positive sputum smears from TB bacilli.

There is a lack of basic homogeneity among the results of the studies conducted all around the world that without respect to the other related genetic factors and environmental factors makes the absolute conclusion very difficult. Therefore, study of other related genes such as; NRAMP1, TNF-α, IL-10, MBL along with environmental factors and synergic effects of them, together with the large population can help to have a better and more complete judgment.

Conclusion

In the present study there was no statistically meaningful relationship between the allele and genotype frequencies of *Fok*I, *Bsm*I, *Apa*I and *Taq*I polymorphisms in the VDR gene and susceptibility to tuberculosis, but when they were analyzed with respect to plasma 25-OHD concentrations, a significant association was seen. The increases in plasma vitamin D levels in individuals (with *Fok*I-ff genotype and low levels of vitamin D) may protect them against active tuberculosis.

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Conflict of Interest

The authors declare no conflict of interest.

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