Association of DC-SIGN and DC-SIGNR Repeat Regions with Susceptibility to Pulmonary Tuberculosis in Zahedan, Southeastern Iran

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Received: 25 Oct. 2015; Accepted: 15 Dec. 2015

Abstract- There are conflicting results concerning *DC-SIGN* and *DC-SIGNR* VNTR polymorphisms. The present study aimed to evaluate the possible association between *DC-SIGN* as well as *DC-SIGNR* VNTR polymorphisms and pulmonary tuberculosis (PTB) in a sample of Iranian population. This case-control study was done on 171 PTB and 161 healthy subjects. The variants were detected by polymerase chain reaction (PCR). *DC-SIGNR* VNTR genotypes in cases were 12.7% for 5/5, 2.4% for 6/5, 32.7% for 7/7, 38.2% for 7/5, 5.5% for 7/6, 1.2% for /5, 0.6% for 9/6, 6.7% for 9/7 in PTB patients and 19.7% for 5/5, 2.0% for 6/5, 31.6% for 7/7, 37.5% for 7/5, 5.7% for 7/6, 0.0% for 9/5, 0.7% for 9/6, 2.6% for 9/7 in controls. The findings showed no significant association between *DC-SIGNR* VNTR polymorphism and PTB. All subjects in cases and controls were 7/7 genotype regarding *DC-SIGN* VNTR polymorphism. Our data propose that *DC-SIGNR* VNTR, as well as DC-SIGN VNTR, were not associated with the risk of PTB in a sample of Iranian population.

Acta Med Iran, 2016;54(5):308-312.

Keywords: Tuberculosis; DC-SIGN; DC-SIGNR; VNTR; Polymorphism

Introduction

Tuberculosis (TB) is a chronic disease, mainly affecting the lungs, and remains a major cause of death worldwide especially in Asia and Africa. One-third of the world's population is infected with *M tuberculosis*, but approximately 5 to 10% of infected subjects develop active TB disease during their lifetimes (1). The reasons why some infected individuals develop active disease not yet fully understood. There is increasing evidence shows that host genetic factors play a major role in TB susceptibility (2-7).

The innate immune system is the first line of host defense against invading pathogens and involves the early recognition and uptake of microbes by host specialized phagocytes such as macrophages and dendritic cells (DCs). Phagocytic cells express a range of cellular receptors recognized as pattern recognition receptors (PRRs), which are involved in the recognizing of microorganisms. One specific PRR-dendritic-cell-specific intercellular adhesion molecule-3 (ICAM-3)-grabbing nonintegrin (DC-SIGN) has been the focus of extensive attention in the context of host–Mycobacterium interactions. Genetic studies on tuberculosis have shown that the genetic polymorphisms, potentially involved in the immune response to tuberculosis, could lead to susceptibility or protection to TB (6,8).

Both DC-SIGN (CD209) and DC-SIGNR (also called *CD209L*) gene is mapped on chromosome 19p13.2-3. DC-SIGN might be an important part of host immunity to TB and one of the candidate genes for the disease susceptibility. DC-SIGN is expressed in macrophages and dendritic cells (DCs) (9). It acts as the main receptor for *M. tuberculosis* in human DCs, and plays an important first line role in host defense against pathogens, by internalization and presentation of the bacterium (10). In mature DCs, DC-SIGN promotes the activation and proliferation of resting T cells and increases primary immune responses (11).

The neck region of *DC-SIGNR* is highly polymorphic, and the polymorphism has been

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investigated for genetic predisposition to various infectious diseases though conflicting results have been reported (12).

Both DC-SIGN and DC-SIGNR (CD209L) have three domains: an N-terminal cytoplasmic region, a neck region containing seven tandem repeats of the 23 amino acid sequence, and a C-terminal domain with homology to C- type lectins. The neck region shows high nucleotide identity between repeats, both within each molecule and between DC-SIGN and DC-SIGNR. It has been shown that this region plays a key role in the oligomerization and support of the carbohydrate-recognition domain, consequently, affecting the pathogen-binding properties of these two receptors (13-15). Recently, we have found a significant association between -336A/G (rs4804803) polymorphism of *DC-SIGN* and PTB (8). In the current study, we aimed to find out the possible association between *DC-SIGN* VNTR as well as *DC-SIGNR* VNTR polymorphism and risk/protection of PTB in a sample of Iranian population.

Materials and Methods

Patients

The present case-control study was performed on 171 PTB patients and in 161 healthy subjects. The local ethics committee of the Zahedan University of Medical Sciences approved the project, and written informed consent was obtained from all participants. All control subjects were from the same geographical origin and were living in the same region as the patients with PTB (Zahedan, southeast Iran).

The diagnosis of PTB was based on clinical symptoms, radiological evidence, and bacteriological investigations such as sputum Acid-Fast Bacillus (AFB) smear positivity, culture and response to antituberculosis chemotherapy (6,8,16,17). Whole blood samples were collected in Na-EDTA tubes from all subjects, and genomic DNA was extracted using salting out as previously described (18).

Genotyping

The VNTR polymorphism of DC-SIGNR was detected using the following pair of primer F: 5`-TGTCCAAGGTCCCCAGCTCCC-3` and R: 5`-GAACTCACCAAATGCAGTCTTCAAATC-3` (19). Into a 20 µl PCR tube, 1 µl of genomic DNA (~100 ng/ml), 1 µl of each primer (10 µM), 10 µl of 2X Prime Taq Premix (Genet Bio, Korea) and 7 µl ddH2O were added. PCR was performed in conditions as follows: 95 °C for 5 min; 95 °C for 30 s, 64 °C for 30 s, 72 °C for 30 s, 30 cycles; 72 °C for 10 min. The PCR products were electrophoresed on 3% agarose gels. 302 bp (allele 3), 371 bp (allele 4), 440 bp (allele 5), 509 bp (allele 6), 578 bp (allele 7), 647 bp (allele 8) and 716 bp (allele 9) (Figure 1). We regenotyped random samples to verify the accuracy of genotyping. No genotyping mistake was found.

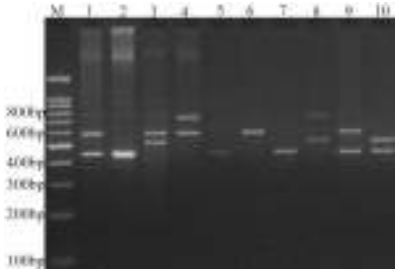

Figure 1. Genotyping of *DC-SIGNR* VNTR polymorphism. Samples were analyzed after separation in a 3% agarose gel electrophoresis. M: DNA Marker; lanes 1, 9: *DC-SIGNR* 5/7; lanes 2, 5, 7: *DC-SIGNR* 5/5; Lane 3: *DC-SIGNR* 6/7; lane 4: *DC-SIGNR* 7/9; lane 6: *DC-SIGNR* 7/7; lane 8: DC-SIGNR 6/9; lane 10: *DC-SIGNR* 5/6.

The *DC- SIGN* repeat region in exon 4 was amplified from genomic DNA with the following pair of primer: forward: 5`-CCACTTTAGGGCAGGAC-3` and reverse: 5`-AGCAAACTCACACCACACAA-3` (19). The polymerase chain reaction (PCR) was carried out in total volume of 20 µL using the following amplification protocol: denaturation at 95°C for 5 min, followed by 30 cycles of 95°C for 30 s, 59°C for 30 s, 72°C for 30 s, with a final extension of 10 min at 72°C. The size of the PCR products of *DC-SIGN* repeat region allele 8 is 922 bp, 7 is 853 bp, and 6 is 684 bp. The PCR products were electrophoresed on 3% agarose, and genotypes were determined (Figure 2).

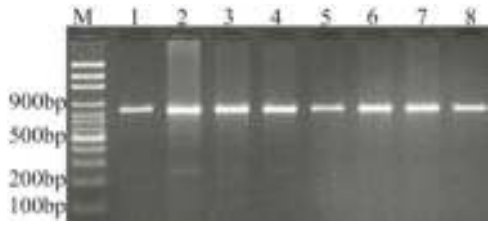

Figure 2. Electrophoresis pattern of PCR product for detection of *DC-SIGN* VNTR polymorphism. M: DNA marker. Lanes 1-8: *DC-SIGN* 7/7.

2.3 Statistical analysis

The statistical analysis of the data was performed using the SPSS 18.0 software. Demographics and biochemical parameters between the groups were

analyzes by independent sample t-test for continuous data and $\chi 2$ test for categorical data. The associations between genotypes and PTB were estimated by computing the odds ratio (OR) and 95% confidence intervals (95% CI) from logistic regression analyzes. *A P*-value less than 0.05 was considered statistically significant.

Results

The study consisted of 171 PTB patients (63 males and 108 females; ages 50.1±20.6 years) and 161 healthy subjects (69 males and 92 females; ages 47.5±15.3 years). There was no significant difference between the groups regarding age and sex (*P*=0.191 and *P*=0.263, respectively).

The genotype and allele frequencies of *DC-SIGNR VNTR* polymorphisms in PTB patients and control subjects are presented in table 1. A total of 8 genotypes and 4 alleles were found for *DC-SIGNR* VNTR polymorphism in our population. We observed a frequency of 12.7% for 5/5, 2.4% for 6/5, 32.7% for 7/7, 38.2% for 7/5, 5.5% for 7/6, 1.2% for 9/5, 0.6% for 9/6, 6.7% for 9/7 in PTB patients, while in controls the genotype frequencies were; 19.7% for 5/5, 2.0% for 6/5, 31.6% for 7/7, 37.5% for 7/5, 5.7% for 7/6, 0.0% for 9/5, 0.7% for 9/6 and 2.6% for 9/7. Neither the overall chi-square comparison of PTB and control subjects ($\chi 2$=7.13, *P*=0.415) nor the logistic regression analysis indicated any association between *DC-SIGNR* VNTR polymorphism and PTB (Table 1). In addition, the allele frequencies were not significant different between the groups ($\chi 2$=5.34, *P*=0.148).

We found that all cases and controls were wild-type genotype (7/7) regarding *DC-SING* VNTR polymorphism, which indicates that the *DC-SIGN* VNTR was not polymorphic in our population.

Table 1. Frequency distribution *of DC-SIGNR* VNTR genotypes and allelics in PTB and healthy subjects (control)

Genotypes	PTB n (%)	Controls n (%)	OR (95%CI)	*P*
5/5	21 (12.7)	30 (19.7)	0.62 (0.32-1.23)	0.223
6/5	4 (2.4)	3 (2.0)	1.19 (0.25-5.57)	0.971
7/7	54 (32.7)	48 (31.6)	Reference	--
7/5	63 (38.2)	57 (37.5)	0.98 (0.58-1.67)	0.978
7/6	9 (5.5)	9 (5.7)	0.89 (0.32-2.42)	0.981
9/5	2 (1.2)	0 (0.0)	4.45 (0.21-95.06)	0.498
9/6	1 (0.6)	1 (0.7)	0.89 (0.05-14.61)	0.942
9/7	11 (6.7)	4 (2.6)	2.44 (0.73-8.19)	0.171
$\chi 2$=7.13, *P*=0.415				
Alleles				
5	111 (33.7)	120 (39.5)	0.80 (0.58-1.12)	0.206
6	14 (4.2)	13 (4.3)	0.94 (0.43-2.05)	0.985
7	191 (57.9)	166 (54.6)	Reference	--
9	14 (4.2)	5 (1.6)	2.43 (0.86-6.90)	0.100
$\chi 2$=5.34, *P*=0.148				

Discussion

DC-SIGN and DC-SIGNR are C-type lectins that serve both as cell adhesion and pathogen recognition receptors. In the present study, we assessed the impact of *DC-SIGN* as well as *DC-SIGNR* VNTR polymorphisms on risk of PTB in a sample of Iranian population. Our findings showed no association between *DC-SIGNR* VNTR polymorphism and PTB in our population. Moreover, the results indicate that the *DC-SIN* VNTR was not polymorphic in our population and all subjects were 7/7 genotypes.

In agreement with our findings, several studies have found no significant differences between TB patients and controls regarding *DC-SIGN* VNTR polymorphism (20-24) and the 7/7 genotype as well as allele 7 is predominant in patients and controls. There is little and controversial studies regarding the possible association between *DC-SIGNR* VNTR polymorphism and vulnerability to TB (21,24). In contrast to our findings, da Silva *et al*., (24) have found that allele with 5 repeats and the heterozygous 6/5 repeats genotype conferred protection to TB development while the allele with 9 repeats associated with increased susceptibility. In agreement with our findings, Barreiro *et al.,* (21)

revealed higher frequencies of 7 alleles and 7/7 genotype in South African TB patients, but none of the *DC-SIGNR* VNTR variants were associated with the TB development. There is no clear explanation for the different findings in different studies. Ethnic, genetic and/or environmental factors might interact in diverse ways to either decrease or increase the susceptibility to tuberculosis in different regions (25).

DC-SIGNR VNTR polymorphism has been shown to be associated with other infections. Xu *et al*., (26) proposed an association between the 9 repeats allele and vulnerability to HIV infection and higher viral loads, in comparison with the alleles 5 repeats. It has been postulated that the presence of 9 repeats alleles could facilitate *M. tuberculosis* entry in the host cell, where it might escape the action of lysosomal enzymes, multiply and affect new cells.(27) In the other hands, receptors with few tandem-repeats could disturb the interaction between the host and the pathogen receptor, leading to prevention of *M. tuberculosis* entry (24).

In conclusion, our results indicate that *DC-SIGN VNTR* and *DC-SIGNR VNTR* were not associated with PTB in a sample of Iranian population. Larger studies with different ethnicities are required to confirm our findings.

Acknowledgement

This work was supported by dissertation grant from Zahedan University of Medical sciences. The authors thank the patients and healthy subjects who willingly participated in the study.

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