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Association between TNF -308G/A polymorphism and susceptibility to pulmonary tuberculosis in the Lur population of Iran

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ABSTRACT

Objective: To investigate whether tumor necrosis factor-α (TNFα) -238G/A and -308G/A polymorphisms are associated with susceptibility to pulmonary tuberculosis (TB) in the Lur ethnic population of Iran.

Methods: TNF polymorphisms genotyping was performed by polymerase chain reaction-restriction fragment length polymorphism method in 100 pulmonary TB patients and 100 healthy controls from the Lur population.

Results: The allelic and genotypic frequencies of TNFα -238G/A polymorphism were not significantly different between the pulmonary TB patients and the healthy controls. However, the TNFα -308G/A polymorphism showed a significantly higher frequency of genotype GG in TB subjects compared to healthy controls (94% in the patients *vs*. 62% in the controls, $P = 0.0001$, odds ratio = 0.104, confidence interval = 0.028–0.382). Moreover, in the TNFα -308G/A polymorphism, a significantly higher frequency of G allele was measured in the patient group compared with the control group (97% in the patient group *vs*. 81% in the control group, $P = 0.0001$, odds ratio = 0.132, confidence interval = 0.038–0.462).

Conclusions: Our findings suggest that TNFα -308G/A polymorphism may increase the susceptibility to pulmonary TB in the Lur population of Iran. Despite TNFα polymorphisms and susceptibility to pulmonary TB, we suggest that more studies with larger sample size are needed in the future. Increasing our understanding of susceptibility risk factors may help to improve current preventive measures and treatment for TB.

1. Introduction

Tuberculosis (TB) is caused by the acid fast bacillus, *Mycobacterium tuberculosis* (MTB). TB is a common infectious disease. Worldwide, there are 9 million novel cases and 2 million mortalities annually [1]. It has been suggested that sensitivity to this disease is variable in the different populations and that contact with this microorganism does not always result in infection. Almost one third of the world population is infected by this bacterium of which 5%–10% are infected with the active form of TB. Additionally, the course and duration of disease vary in different individuals [2].

These differences may be due to host factors and genetic sensitivity of different individuals to this disease [3,4]. Different genetic factors are implicated in the susceptibility to and severity of TB, one of which is *KIR3DS1* gene and it combines with HLA-B Bw4 and Ile80 ligand [5]. Moreover, human and mouse studies on MTB infection have demonstrated different loci in the susceptibility or resistance to TB such as toll like receptors [6–10]. It has been reported that tumor necrosis factor (TNF) is involved in the prevention of mycobacterial infection development and also in preventing progression from latent to active TB form [11–14].

TNFα is a cytokine involved in the innate immunity. It is primarily formed against pathogens, and its genetic polymorphisms play an important role in the immune response efficacy against major infections. The principal source of TNFα production is the mononuclear phagocytes. The release of TNFα

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from these cells results in the recruitment of neutrophils and monocytes to the site of infection [15].

The effect of TNFα on the human body varies from activation of inflammatory processes to activation of hepatocytes and tissue damage based on its low, moderate and high doses. It also contributes to activation of macrophages and regulates interferonγ production [16]. Therefore, it initiates pro-inflammatory reactions implicated in the effect on disease and resistance to the *Mycobacterium* [17].

The human and animal TB investigations suggest that TNFα impresses the innate immune response to TB [18]. Hence, TNFα -238G/A and -308G/A polymorphisms have been analyzed widely as the nominated genes for susceptibility to TB in different populations [19–23]. Due to the differences between distribution of TNFα gene in different races and nations and also due to association of TNFα polymorphisms with pulmonary TB susceptibility, we were interested to investigate the prevalence of these polymorphisms in pulmonary TB patients and healthy controls of the Lur population dwelled in Lorestan Province. Therefore, the susceptibility to pulmonary TB infection was ascertained by the studying of TNFα -238G/A and -308G/A polymorphisms in the TB group and the results were compared with the healthy control group.

2. Materials and methods

2.1. Patients and controls

This study was approved by the Ethics Committee of Lorestan University of Medical Sciences. All subjects gave informed written consent. We used case control study to implement this investigation. The patient group was comprised of 100 unrelated Lur individuals referred to the health center of Khorramabad city of Lorestan Province, with TB confirmed by sputum culture. All patients received TB standard treatment and none of them had drug resistance. The control group was comprised of 100 unrelated Iranian individuals of the same race and geographic region. The controls were asymptomatic, had normal radiologic results and their purified protein derivatives test was negative. The control group was matched on age and sex with the patient group. Additionally, all study subjects had parents of the same race. The blood samples were collected for analysis.

2.2. Genotyping

Patient and control DNA samples were extracted using QIAmp kit (Qiagen, Germany). The polymerase chain reaction-restriction fragment length polymorphism previously suggested by Fan *et al*. was performed to determine TNFα -238G/A and -308G/A polymorphisms in patients and controls using their genomic DNA [24]. The list of forward and reverse primer sequences (Qiagen, Germany), restriction enzymes (Biolabs, USA) and digestion patterns for different alleles were assorted in Table 1.

The amplification was carried out by using Mastercycler (BioRad, USA) in 20 μL reaction. Amplification conditions used were as follows: denaturation initiated at 94 °C for 5 min and was followed by 5 cycles of denaturation at 94 °C for 5 min, annealing at 60 °C for 1 min, extension at 72 °C for 1 min, and 25 cycles of denaturation at 94 °C for 30 s, annealing at 60 °C for 30 s, extension at 72 °C for 40 s with final extension at 72 °C for 1 min. The PCR products were incubated with restriction enzymes Msp I and Nco I at 37 °C for TNFα -238G/A and -308G/A, respectively for 24 h to digest the DNA. The electrophoresis of PCR products was accomplished on 3% agarose gel consisting of 0.5 mg/mL ethidium bromide. Finally, the products were visualized by using an ultraviolet light.

2.3. Statistical analysis

The genotypic and allelic frequencies of TNFα -238G/A and -308G/A polymorphisms were ascertained by direct counting in the TB population and healthy control population. All polymorphisms were consistent with values predicted by Hardy–Weinberg equilibrium in both patient and control groups. The differences in the genotypic and allelic frequencies of TNFα -238G/A and -308G/A polymorphisms were determined by the *Chi*-squared test and Fisher's exact test between TB population and healthy control population. Overall, $P < 0.05$ was considered statistically significant after correction. The odds ratio (OR) was calculated by the cross-product ratio and exact confidence interval (*CI*) of 95% was obtained.

3. Results

The study subjects comprised of 100 healthy controls with the mean age, (30.21 ± 2.55) years and 100 pulmonary TB

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

TNF-α polymorphisms	Genotypes	Associated phenotypes	% of TB patients group	% of Healthy controls group
TNF (-238G/A)	GG		96	92
	GA		4	8
	AA		–	–
TNF (-308G/A)	GG	Low	94*	62
	GA	Intermediate	6	38
	AA	High	–	–

*: Significant difference ($P < 0.05$), $n = 100$.

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

TNF-α polymorphisms	Sequences of the primers	PCR product size	Restriction enzymes	Length of the restriction fragments for different alleles
TNF (-238G/A)	F: 5′ AGAAGACCCCCCTCGGAACC 3′ R: 5′ ATCTGGAGGAAGCGG TAGTG 3′	152 bp	Msp I	G-133 bp + 19 bp A-152 bp
TNF (-308G/A)	F: 5′ AGGCAATAGGTTTTGAGGGCCAT 3′ R: 5′ ACACTCCCCATCCTCCCT GCT 3′	117 bp	Nco I	G-97 bp + 20 bp A-117 bp

F: Forward; R: Reverse; bp: Base pairs.

Table 3
Distribution of TNF-α alleles in TB patients group and healthy controls group.

TNF-α polymorphisms	Alleles	% Allele frequency in TB patients group	% Allele frequency in healthy controls group
TNF (-238G/A)	G	98	96
	A	2	4
TNF (-308G/A)	G	97*	81
	A	3	19

*: Significant difference ($P < 0.05$).

patients with the mean age, (39.65 ± 3.87) years. Among the healthy controls, 50 individuals were males and 50 individuals were females, and among the pulmonary TB patients, 40 individuals were males and 60 individuals were females.

The genotypic and allelic frequencies of TNFα -238G/A and -308G/A polymorphisms were listed in Tables 2 and 3. The genotypic and allelic frequencies of TNFα -238G/A polymorphism did not have significant difference between the pulmonary TB patients and the healthy controls. Also, AA genotype of TNFα -238G/A polymorphism was not detected in the patient and control groups. Only, in TNFα -308G/A polymorphism, a significantly increased frequency of genotype GG was observed among patients compared with controls (94% in the patient group *vs*. 62% in the control group, $P = 0.000\,1$, OR = 0.104, *CI* = 0.028–0.382). Also AA genotype of TNFα -308G/A polymorphism was not observed in the patient and control group (Table 2).

In the TNFα -308G/A polymorphism, a significantly increased frequency of G allele was observed among the patient group compared with the control group (97% in the patient group *vs*. 81% in the control group, $P = 0.000\,1$, OR = 0.132, *CI* = 0.038–0.462) (Table 3).

4. Discussion

TNFα is one of the most significant cytokines involving in the primary host innate immunity against a pathogen. Moreover, it is shown that TNFα is characteristic in the prevention of mycobacterial establishment and preservation of TB in latent form [11–14]. Among this, TNFα -238G/A and -308G/A polymorphisms were widely determined as the nomination genes in the susceptibility to TB infection [19–23].

In this study, we showed the effect of TNFα -238G/A and -308G/A in the susceptibility to pulmonary TB in the Lur population of Iran. The genotypic and allelic frequencies of TNFα -308G/A polymorphism have significant difference between patients infected by pulmonary TB and the healthy control individuals. The TNFα -238G/A polymorphism had no significant difference between pulmonary TB patients and the healthy control group. In contrast, in the late studies carried out by Sharma *et al*. [12] and Kumar *et al*. [21] in the north of India, there were no association between these polymorphisms and pulmonary TB. This phenomenon is not surprising because of genetic nonidentity between Asians and Indians (racial differences).

Our results showed similar outcome as former studies in this field conducted by Fan *et al*. [24] and Qu *et al*. [25] on the Asian population which indicated the association of TNFα -308G/A with TB. One possible explanation for this is the genetic similarities between Asian populations. The study by Ben-Selma *et al*. [26] also indicated the association between TNFα -308G/A polymorphism and TB in the Tunisian population. Also, study by Amirzargar *et al*. [27] has demonstrated the association of TNFα -238G/A polymorphism with TB in other ethnic group of Iranian.

TNFα is an essential cytokine in the granuloma formation. The mice with deficiency in TNFα are impotent in the granuloma formation. This tragedy leads to MTB development and fulminant death in the infected animals. Furthermore, the former studies have demonstrated that TNFα blocking can lead to TB reactivation [28,29]. Hence, despite of paradoxical findings, the association of TNFα polymorphisms with TB seems undeniable. However, we can indicate other TNFα polymorphisms, the race of studied population and the size of studied samples as the reasons of these adverse results.

Finally, our findings show that TNFα -308G/A polymorphism may associate with pulmonary TB in the Lur population of Iran. Despite susceptibility to pulmonary TB, we suggest that more studies with larger sample size should be carried out to verify the role of TNFα polymorphisms, especially TNFα -308G/A polymorphism in the future.

Conflict of interest statement

We declare that we have no conflict of interest.

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