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Evaluation of TNF-alpha gene (G308A) and MBL2 gene codon 54 polymorphisms in Turkish patients with tuberculosis

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ABSTRACT

Objective: MBL acts as a binding protein that enables uptake of mycobacteria into macrophages. And, TNF-alpha is an important cytokine that is involved in control of mycobacterial infections both in-vivo and in-vitro. A large number of genetic factors exerting susceptibility to tuberculosis has been identified, among which mannose-binding lectin and tumor necrosis factor-alpha call attention. The objective of this study is to compare the frequency of TNF-alpha and MBL gene polymorphisms between patients diagnosed with tuberculosis and healthy volunteers in Turkey, and determine the association between tuberculosis and TNF-alpha gene (G308A) and MBL2 gene codon 54 polymorphisms.
Material and methods: The study included 69 patients who were diagnosed with tuberculosis and 70 control subjects. The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was used to detect TNF-alpha (G308A) gene and MBL2 gene codon 54 polymorphisms. For statistical analysis, the significance level was determined as $p < 0.05$.
Results: A comparison between patient and control groups in TNF-alpha (G308A) gene and MBL2 gene codon 54 polymorphisms showed no statistically significant difference ($p > 0.05$). However, a comparison of mean body mass index (BMI) and smoking status showed a statistically significant difference between the tuberculosis and control groups ($p = 0.01$ and $p = 0.009$, respectively).
Conclusion: Our results suggest that the MBL2 gene Codon 54 and TNF-alpha gene G308A polymorphisms are not associated with an increased risk for development of tuberculosis in our patients. Further studies are required including more cases of tuberculosis patients and other potentially relevant gene polymorphisms.

Introduction

Although almost all of the individuals infected with *Mycobacterium tuberculosis* develop infections, only 5–10% of those with infections present with an active disease [1]. On the other hand, only a very small number of patients have an underlying risk factor such as diabetes mellitus, advanced age, alcohol abuse, HIV infection, and corticosteroid usage [2]. Until recently, environmental factors such as socioeconomic status, malnutrition and crowded housing have been implicated for that. However, numerous studies ranging from family and twin studies to novel molecular methods conducted during the last decade show that genetic factors of the host are at least as important as the environmental factors [3,4].

Mannose binding lectin (MBL) acts as a highly protected antibody that can efficiently bind to many sugars. Since most of the sugars it binds to are uncommon in mammalian cells at high densities, it does not usually recognize self determinants, and it often adopts well to the surfaces of microbial cells. Binding to these cells result in phagocytes' adherence to bacteria coated with MBL, ingestion and killing of these bacteria in the cell. Therefore, MBL acts as an opsonizer. MBL deficiency, which is the most common global immune deficiency, is associated with recurrent childhood infections in children [1]. *M. tuberculosis* contains lipoarabinomannan and phosphatidylinositol mannoside in the cell wall. Since each of these molecules are mannose-containing carbohydrates, they can be bound by MBL. MBL functions like a binding protein that enables the uptake of mycobacteria into macrophages. Thus, it is suggested that functional polymorphisms associated with low levels of MBL in serum may contribute to susceptibility to the tuberculosis [5]. At present, three structural polymorphisms of MBL2 gene have been

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Table 1
Clinical and demographic characteristics of patient and control groups.

	Tuberculosis patient group n = 69	Control group n = 70	p value
Gender (Male/Female)	47/22	45/25	0.633
Age (years ± SD)	37.8 ± 12.7	39.4 ± 10.8	0.213
BMI (kg/m^2)	23.1 ± 4.7	25.7 ± 4.1	0.010
Smoking status (Yes/No)	22/47	35/35	0.009
Smoking, pack years	12.7 ± 2.5	7.5 ± 1.5	0.081
BCG scar (+)	44 (63.8%)	49 (70%)	0.850
BCG scar (−)	25 (36.2%)	21 (30%)	
Pulmonary tuberculosis	48 (69.6%)		
Non-pulmonary tuberculosis	21 (30.4%)		

Table 2
Distribution of genotypes between the patient and control groups.

	Tuberculosis patient group n = 69 (%)	Control group n = 70 (%)	p value
MBL2 gene codon 54 polymorphism (rs1800450, c.161G >A)			
G/G genotype	48 (69.6)	47 (67.1)	0.62
G/A genotype	13 (18.8)	11 (15.7)	
A/A genotype	8 (11.6)	12 (17.1)	
G Allele	109 (79.99)	105 (75.00)	0.43
A Allele	29 (21.01)	35 (25.00)	
TNF-a gene G308A polymorphism			
G/G genotype	56 (81.2)	60 (85.7)	0.19
G/A genotype	13 (18.8)	8 (11.4)	
A/A genotype	0 (0)	2 (2.9)	
G Allele	125 (90.58)	128 (91.43)	0.80
A Allele	13 (9.42)	12 (8.57)	

identified for MBL deficiency. All of these three polymorphisms are located in a short segment of the first exon of the gene, resulting in a single amino acid change in the collagenous part of the protein. Polymorphisms at codon 54 and 57 disrupt the secondary structure of the protein, and they are associated with reduced serum MBL levels. The polymorphism at codon 52 has no impact on the serum levels of the protein. Studies suggest increased rate of infection for polymorphic alleles in the MBL2 gene in both homozygous and heterozygous individuals [6].

TNF-a is a cytokine which is involved in many inflammatory and immune-mediated responses. It is mainly secreted from other cells stimulated by monocytes, macrophages, T and B lymphocytes, natural killer cells or microbial products. It is required for induction of the cytokine cascade in order to provide immune response [7]. So far, there has been a few major polymorphisms identified in the TNF locus [8–10]. Among them, LT-a +250 and TNF-alpha-308 polymorphisms have been associated with increased basal and stimulated TNF-alpha expression. Therefore, these polymorphisms have been associated with susceptibility to many inflammatory and infectious conditions [8].

The objective of the present study was to examine TNF alpha and MBL gene polymorphism between patients with tuberculosis and control groups in Turkey and evaluate the association between TNF alpha and MBL gene polymorphism and development of tuberculosis.

Material and methods

Study subjects

The study included a total of 69 patients who were treated for tuberculosis and a total of 70 healthy control subjects. The diagnosis of tuberculosis patients was based on sputum smear positivity, sputum culture positivity as well as histopathological and clinical-radiological examinations. A particular attention was paid for healthy control group to ensure that they had a negative history of tuberculosis, no comorbid disease and a normal PA chest X-ray. For study and healthy control groups, information including age, sex, body mass index, average monthly income, history of smoking, presence of familial tuberculosis, comorbidity, presence of BCG scar and history of medication use were recorded, and type and site of tuberculosis, and diagnostic modality used were evaluated. Patients with any chronic comorbidity were excluded. The study protocol was approved by the Local Ethical Committee of Uludag University.

DNA isolation and genotyping of MBL2 and TNF-a

The venous blood samples obtained from the patients and control subjects were collected in EDTA tubes. Genomic DNA was extracted from whole blood using a DNA isolation kit (Dr Zeydanlı Life Science Ltd., Ankara, Turkey) according to the manufacturer's instructions, and samples were stored at −20 °C until PCR analysis.

The MBL2 gene codon 54 polymorphism (allele B: rs1800450, c.161G >A; p.54Gly >Asp) was determined using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. For the MBL2 gene codon 54 polymorphism, forward (5′-TAGGACAGAGGGCATGCTC-3′) and reverse (5′-CAGGCAGTTTCCTCTGGAAGG-3′) primers were used to amplify a 349 bp region of the MBL2 gene [11]. To identify the MBL2 gene codon 54 polymorphism among the products, the Ban I (Genemark, Taiwan) enzyme was used. The restriction fragments were separated using a 2% agarose gel. Genotypes were determined as follows: genotype A/A was two distinct products of 260 bp and 89 bp; genotype A/B was three distinct products of 349 bp, 260 bp and 89 bp; and genotype B/B was one 349 bp fragment. For MBL2 gene codon 54 polymorphism, the normal allele is called A, and the variant allele is called B.

The genotypic analysis of the TNF-alpha gene G308A polymorphism was performed using a modified version of a previously described PCR-restriction fragment length polymorphism (RFLP) assay [12]. Briefly, G308A genotyping was conducted using the forward primer 5′-AGGCAATAGGTTTTGAGGGCCAT-3′ and the reverse primer 5′-TCCTCCCTGCTCCGATTCCG-3′, which enabled the use of the restriction enzyme NcoI. PCR products were digested with the restriction enzymes NcoI (Genemark, Taiwan) at 37 °C for 16 h and

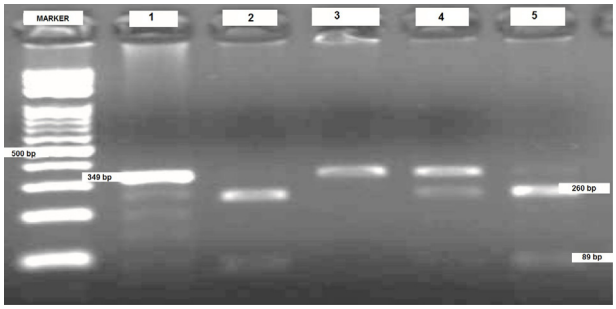

Fig. 1. Photograph of the PCR products of the MBL2 gene gene after Ban I enzyme cutting and on 2% agarose gel. Line MARKER shows the 100 bp DNA ladder, lines 1 and 3 show individuals with A/A genotype (349 bp), line 2 and 5 show G/G genotype (260 bp, 89 bp), and line 4 shows the G/A genotype (349 bp, 260 bp, 89 bp).

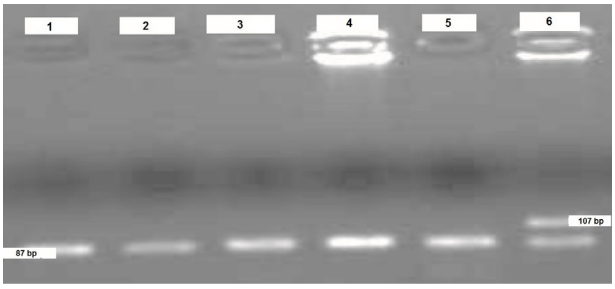

Fig. 2. 4% agarose gel electrophoresis of Nco I digested PCR products for genotyping the TNF-alpha gene G308A polymorphism. Line 1, 2, 3, 4 and 5 show individuals with G/G genotype (107 bp), and line 6 shows individuals with G/A genotype (107 bp, 87 bp).

analyzed on a 4% agarose gel. When G was present at codon 308, the 107 bp PCR product was cleaved into two fragments of 87 bp and 20 bp.

Statistical analysis

Continuous variables were presented as the mean + standard deviation, and the range (min–max values). The comparison between groups for continuous variables without normal distribution was performed using the Mann–Whitney U test, and the changes in frequency were calculated for appropriate variables. Pearson's chi-square and Fisher's exact tests were used to test the distribution of categorical variables between groups. A p level of <0.05 was considered significant.

Results

Among 69 cases in the patient group (47 males and 22 females), the mean age was 37.8 ± 12.7 years while it was 39.4 ± 10.8 years among 70 cases in the control group (45 males and 25 females). There was no difference in age and gender between the patient and control groups. The average body mass index (BMI) and smoking status were significantly different between the patient group with OSAS and the control group ($p = 0.01$, and $p = 0.009$, respectively). Other characteristics are shown in Table 1.

Evaluation of the MBL2 gene codon 54 polymorphism (allele B: rs1800450, c.161G >A; p.54Gly >Asp) revealed that in the patient group, 48 (69.6%) had the G/G genotype, 13 (18.8%) had the G/A genotype, and 8 (11.6%) had the A/A genotype. In the control group, 47 (67.1%) had the G/G genotype, 11 (15.7%) had the G/A genotype and 12 (17.1%) had the A/A genotype. With respect to genotype distribution, no significant difference was observed between the tuberculosis patients and the controls ($p = 0.62$). The frequency of A allele was 21% in the patient group and 25% in the control group, with a similar rate in both groups. No significant difference was observed in the frequencies of A allele between the patient and control groups ($p = 0.43$) (Table 2).

Evaluation of the TNF-alpha gene G308A polymorphism revealed that in the tuberculosis patient group, 56 (81.2%) had the G/G genotype, 13 (18.8%) had the G/A genotype, and (0%) had the A/A genotype. In the control group, 60 (85.7%) had the G/G genotype, 8 (11.4%) had the G/A genotype, and 2 (2.9%) had the A/A genotype. With respect to genotype distribution, no significant difference was observed between the tuberculosis patients and the controls ($p = 0.19$). The frequency of A allele was 21% in the patient group and 25% in the control group, with a similar rate in both groups. No significant difference was observed in the frequencies of A allele between the patient and control groups ($p = 0.80$) (Table 2).

Discussion

M. tuberculosis is the main cause of tuberculosis, which remains a major global infectious disease, and almost all of the individuals who are infected with *M. tuberculosis* develop infections, but only up to 5–0% have active disease. Only a small number of these patients have an identifiable underlying risk factor. Recent studies have shown that genetic factors of the host are involved in this condition as much as the socioeconomic factors and environmental factors including crowded housing and malnutrition [1–4].

MBL is a calcium dependent plasma collectin, which plays an important role in natural immune defense against infectious agents [6]. Mannose binding lectin has also an important role in inherited immunity. Recent studies on the relation between MBL genes and TB have shown different, and even contradictory results. A meta-analysis by Denholm et al. reported that MBL gene polymorphism was associated with serum levels of MBL, but not with development of tuberculosis. MBL levels are elevated in the setting of tuberculosis infection. Although these high MBL levels indicate protection against TB infection, an elevated level of MBL is reported to be consistent with the acute phase reaction [13] (Fig. 1).

An Indian study found that functional polymorphic homozygotes for MBL were more prevalent in tuberculosis patients compared to the control group. They showed that polymorphisms of the MBL2 gene are associated with lower serum MBL levels that is, in turn, related with recurrent infections during childhood and probably adulthood [5]. A study in West African population reported that a variant of the MBL2 gene at codon 57 is involved in protection against tuberculosis [14]. They suggested that although MBL2 gene codon 57 is associated with tuberculosis in India, it might provide protection against tuberculosis in Africa. A study in patients with tuberculosis meningitis found that MBL2 gene at codon 54 polymorphism confers a strong protection against tuberculosis meningitis. Lower MBL levels are considered to prevent extrapulmonary spread of tuberculosis, with a potential protection against tuberculosis meningitis [15]. A study by Cosar et al. [16] in children found that the frequency of AB genotype which produces lower MBL levels was significantly lower in tuberculosis patients compared to the control group. The difference between the extrapulmonary group and healthy controls was especially significant. These results indicated that low levels of MBL and AB genotype confers protection against tuberculosis, particularly extrapulmonary tuberculosis in children. Another study found no relation between tuberculosis and MBL2 gene polymorphism in Turkish children [17]. The difference in these studies can be explained by two facts. Firstly, development of the disease may be associated with genetic factors of the host and pathogen as well as the environmental factors, or multiple contributing genetic factors might have played a role in development of susceptibility by individuals to specific infections. Our study has shown that there is no significant differ-

ence in MBL2 gene polymorphism in cases of adults diagnosed with tuberculosis ($p > 0.05$) (Fig. 2).

Susceptibility to infectious diseases is influenced by genetic background, and a well functioning cellular immune activation is responsible for protection. In tuberculosis, TNF-a is a central mediator of granuloma formation, and it controls the spread of bacilli, acting synergistically with IFN-y, and thus preventing infection by *M. tuberculosis*. A Dutch study by Jujjermans et al. [18] found that TNF receptor 1 and 2 concentrations were higher in the group of tuberculosis patients compared to controls, and they were reduced by treatment. Single nucleotide polymorphisms of TNF genes may have an impact on cytokine levels, mediating the resistance and susceptibility against tuberculosis. A study in tuberculosis patients and their household contacts examined the TNF-α (-308G/A) genotype polymorphism, and found no difference in TNF-α gene polymorphism between these two groups [19]. Studies in India on the presence of single nucleotide polymorphism of TNF-α gene promotor -308 G > A have reported contradictory results [20,21]. In a meta-analysis, Pacheco et al. [22] found no association between TNF-alpha-308G/A polymorphism and tuberculosis. Studies in Turkey on the presence of polymorphism in this gene region showed no statistically significant association similar to our study [23–25].

However, some other studies produced different results. A study by Bikmaeva et al. [26] found that frequency of allele TNF2 was significantly higher in tuberculosis patients than in control group. They suggested presence of an association between this TNF allele and pulmonary tuberculosis. Another study showed that the number of 308 GG TNF homozygous individuals in tuberculosis group was significantly lower than those in control group [27]. Another study performed a haplotype analysis with HLA and TNF-alpha and beta gene polymorphisms in pulmonary tuberculosis. And they found no difference in genotype frequencies of TNF-alpha-238, and -308 and TNF-beta between patient and control groups. However, they showed that in combination with the HLA genes/gene products such as HLA-A1, B17, B21 and DR7, the TNF-alpha and beta genes as haplotypes are associated with protection against the disease as well as an increased susceptibility to bacteriological relapse [28].

Some limitations of our study include smaller number of cases compared to other studies and lack of measurement of serum MBL levels seen in other studies along with genetic analysis.

In conclusion, no significant difference was found between tuberculosis patients and controls in terms of MBL2 gene codon 54 and TNF-alpha gene G308A polymorphisms. It can be suggested that the MBL2 gene codon 54 and TNF-alpha gene G308A polymorphisms may not confer a role in susceptibility to tuberculosis in Turkish patients. Future studies are needed in Turkish patients involving large sample sizes and other TNF-alpha and MBL2 gene polymorphisms as a disease susceptibility factor in tuberculosis.

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Competing interests

None declared.

Ethical approval

Not required.

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