The effect of mannose-binding protein gene polymorphisms in recurrent respiratory system infections in children and lung tuberculosis

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SUMMARY: Özbaş-Gerçeker F, Tezcan İ, Berkel Aİ, Özkara Ş, Özcan A, Ersoy F, Sanal Ö, Özgüç M. The effect of mannose-binding protein gene polymorphisms in recurrent respiratory system infections in children and in lung tuberculosis in adults. Turk J Pediatr 2003; 45: 95-99.

Mannose-binding lectin (MBL) is able to bind pathogens as an opsonin and plays an important role in the innate immunity. The aim of the present study was to determine the frequencies of the MBL gene variants in the Turkish population and to examine the presence of any association between MBL variants and development of tuberculosis (TB) in adults and recurrent respiratory tract infections in children. Two structural gene mutations in exon 1 of MBL gene (codon 54 and codon 57) were studied. The overall distribution of genotypes did not significantly differ between controls and TB patients/children with recurrent respiratory system infections.

The frequency of allele B was calculated as 0.14, 0.09 and 0.06 for control, TB patients and children with recurrent respiratory system infections, respectively. It was found to be significantly lower in children with recurrent respiratory system infections than in controls (χ^2: 4.68, d.f: 1, p: 0.030).

Key words: mannose-binding lectin, tuberculosis, recurrent respiratory system infections.

Mannose-binding lectin (MBL) is a liver-produced C-type serum lectin, which is thought to play an important role innate immunity. It is also found in nasopharyngeal secretions, middle ear fluid, inflammed joints, amniotic fluid and serum[1]. MBL is a member of the collectin family characterized by the presence of a carbohydrate recognition site, and it is able to bind pathogens carrying mannose and N-acetyl glucosamine residues as an opsonin. It can activate the MBL pathway of the complement by mediating phagocytosis and using MBL serine proteases 1 and 2[2]. MBL shows a modular domain composition and three-dimensional structure similar to C1q, which is a member of the complement system[3]. The protein is composed of six subunits, each consisting of three identical 32-kDa polypeptide chains[4].

Human MBL gene has been mapped to chromosome 10q11.2-q21[5]. The gene comprises four exons which encode a cysteine-rich region (exon 1), a collagenous region (exon 1 and 2), a "neck" region (exon 3) and a carbohydrate recognition site (exon 4). Three structural gene mutations in the first exon of the gene (codon 52, codon 54 and codon 57) were found to be associated with low serum levels of MBL[6]. Mutation at codon 54 results in a change of glycine to aspartic acid (allele B), at codon 57 glycine to glutamic acid (allele C), and codon 52 arginine to cysteine (allele D), and the normal MBL allele is designated by allele A[7]. Three mutations impair the assembly of mannose binding protein homopolymer[8] which makes the subunits more vulnerable to degradation and reduces the amount of functional MBL subunits. Madsen et al.[9] found additional polymorphisms in the promoter region of MBL gene. It is also suggested that there may be additional undetected polymorphisms governing MBL production or

another external factor that may influence the transcriptional regulation of the gene[10].

The frequencies of three structural gene mutations have been studied in different population groups. Codon 54 mutation has been identified in Eurasian populations at a frequency range of 0.11-0.16. Codon 57 mutation seems to have higher frequencies in sub-Saharan African populations (0.23-0.29), and codon 52 mutation occurs at much lower frequencies in African than non-African populations[11].

The associations of MBL gene variants with several diseases have been studied[12-16]. Garred et al.[17] proposed that homozygous carriers of variant MBL alleles are at increased risk of HIV infection due to high susceptibility to co-infections. Furthermore, they suggested alleles are associated with significantly shorter survival time after a diagnosis of AIDS. The influence of the presence of structural MBL variant alleles in the course of lung disease and on survival in patients with cystic fibrosis (CF) has been studied, and the presence of MBL variant alleles was found to be associated with poor prognosis and early death[18].

A striking association between susceptibility to severe recurrent respiratory system infections and the codon 54 mutation was reported in British families[8].

It has been hypothesized that high serum concentrations of MBL may facilitate the entrance of certain pathogens, especially intracellular microorganisms, or increase their pathogenicity[17]. MBL allele B (G54D) was found to confer protection against tuberculous meningitis[19]. A significant association between MBL B allele and protection against the development of meningeal tuberculosis was found and it was hypothesized that individuals with lower levels of MBL appear to be protected to some extent against developing tuberculosis (TB). It was thought that MBL protects against infection with encapsulated organisms, but it may also facilitate the uptake and survival of intracellular pathogens such as TB within macrophages and the intracellular tropism of the mycobacterium within macrophages.

The aim of the present study was to determine the frequencies of the MBL variants in the Turkish population and to examine the presence of any association between MBL variants and development of tuberculosis in adults and of recurrent respiratory tract infections, in children.

Material and Methods

Patients

Forty-nine patients (aged between 27 and 47 years old) with lung tuberculosis and 69 children (aged between 6 months and 3 years old) with recurrent otitis media (3>) without well defined primary immunodeficiency disorder were included in this study. Informed consent was obtained from the parents of the children and from the patients with tuberculosis.

Healthy Control Subjects

Blood samples from unrelated individuals 25-45 years of age (n:100) were randomly obtained from different geographical regions of Turkey and constitute a repository at the TUBİTAK DNA/Cell Bank & Gene Research Laboratory.

DNA Isolation

Ten milliliter peripheral blood samples were taken into EDTA tubes. Genomic DNA was isolated from these samples according to standard procedure[20]. DNAs were stored in TE buffer (pH. 7.5) at 80°C until use.

Polymerase Chain Reaction (PCR) Amplification of MBL Gene Exon 1

Exon 1 of MBL gene was amplified by PCR. The primer sequences were 5'-dGTAGGAC-AGAGGGCATGCTC-3' and 5'-dCAGGCAGTT-TCCTCTGGAAGG-3'. For the analysis of codon 54 mutation, 328 bp product was digested by 5u BanI, and for the codon 57 mutation the same product was digested by 3.5 u MboII at 50°C for 60 min and at 37°C for 90 minutes. After enzyme digestion, the resulting products were visualized by electrophoresis on 2% agarose gels.

Statistical Analysis

The frequencies of the MBL variants were calculated according to the Hardy-Weinberg Law. The comparison of genotype frequencies between recurrent respiratory system

infections/tuberculosis and MBL genotypes was done by 3x2 chi-square test and the difference in the allele frequencies was tested using 2X2 chi-square analysis. Odds ratio with 95% confidence interval (CI) was calculated to approximate the risk of disease.

Results

The codon 54 and 57 mutation of the MBL gene were studied in children with recurrent respiratory system infections, patients with tuberculosis and healthy control subjects. Allele C was not observed in any group and therefore the frequency of codon 57 mutation was 0.00 for all groups.

The comparison of the MBL codon 54 genotype and allele frequencies between TB patients and the control subjects is given in Table I. The overall distribution of genotypes did not significantly differ between TB patients and controls (χ^2:2.14, d.f:2, p:0.34). The frequency of the A/A genotype compared with A/B+B/B was not significantly different between the two groups (χ^2:0.32, d.f:1, p:0.57). There was no significant difference in the allele frequency of codon 54 wild type (allele A) between patients and controls (χ^2:1.40, d.f:1, p:0.24).

Table II shows the genotype and allele frequencies of MBL variants in children with recurrent respiratory system infections and control subjects. No significant difference was absorved in the overall distribution of genotypes between patients and controls (χ^2:4.16, d.f:2, p:0.125). The frequency of A/A genotype compared to A/B+B/B was not significantly different between the two groups (χ^2:3.32, d.f: 1, p:0.068). On the other hand, the frequency of B allele was found to be significantly lower in children with recurrent respiratory system infections than in controls (χ^2:4.68, d.f:1, p: 0.30, OR:2.33, 95% CI:1.06-5.11).

Table I. Comparison of Genotype and Phenotype Frequencies of MBL Variants Between Patients with Lung Tuberculosis (TB) and Control Subjects

	TB patients (n:49)		Control (n:100)		χ^2	p
Genotypes						
A/A	40	0.82	76	0.76		
A/B	9	0.18	20	0.20	2.14	
0.34						
B/B	0	0	4	0.4		
Alleles						
A	89	0.91	172	0.86		
					1.40	0.24
B	9	0.09	28	0.14		

MBL: mannose-binding lectin.

Table II. Comparison of Genotype and Phenotype Frequencies of MBL Variants Between Children with Recurrent Respiratory System Infections (RI) and Control Subjects

	Children with RI (n:69)		Control (n:100)		χ^2	p
Genotypes						
A/A	61	0.88	76	0.76		
A/B	7	0.10	20	0.20	4.16	
0.125						
B/B	1	0.01	4	0.4		
Alleles						
A	129	0.93	172	0.86		
					4.68	
0.030						
B	**9**	**0.06**	**28**	**0.14**		

Discussion

The aim of the present study was to analyze two mutations in exon 1 of MBL gene (codon 54 and codon 57) in Turkish control subjects, patients with tuberculosis and children with recurrent respiratory system infections in order to determine the frequencies of these mutations in our population and to investigate a possible modifying role of the MBL gene variants in tuberculosis and recurrent otitis media.

The frequency of the codon 57 mutation was found to be zero for all groups. This was a predicted result since this mutation is mostly specific to South African populations. It indicates a meaningful genetic difference of our population from the African populations with respect to this locus.

The frequency of the codon 54 mutation (allele B) in Turkish control subjects was found to be 0.14, which is within the range of European populations.

The association between MBL variants and susceptibility to several diseases has been reported. Hoal-van Helden et al.[19] studied the contribution of MBL to susceptibility to TB or to progression of disease in a well defined South African population. A

significant association was found between allele B and protection against the development of meningeal TB. In our study, we could not find any significant association between MBL genotypes and TB. The frequency of the codon 54 mutation in the TB group was lower than that of the control group. Although it was found statistically insignificant, this may suggest that allele B has a role in the protection against tuberculosis.

We have also studied the association between MBL variants and recurrent otitis media in children. No significant difference was observed in the overall distribution of the genotypes. As an unexpected result, the frequency of allele B was significantly lower in children with recurrent respiratory system infections than in control subjects. We conclude that it may be helpful to test the association between MBL variant genotypes and other infectious diseases in a large series of patients in order to resolve the role of MBL protein in susceptibility to or protection against infections with different pathogens in certain age groups.

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