Association between mannose binding lectin polymorphisms and predisposition to bacterial meningitis

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The aim of this study was to examine the presence of any association between mannose binding lectin (MBL) gene variants and bacterial meningitis. Codon 54 (B allele) and codon 57 (C allele) polymorphisms in exon 1 of the MBL gene were investigated in 50 healthy controls and 31 patients diagnosed as purulent meningitis. Codon 57 polymorphism was not found in our patient and control groups. B allele frequency was significantly higher in the patient group (22%) compared to the control group (3%). AB genotype was determined in 39% and 6% of patient and healthy control groups, respectively, and the difference was statistically significant. AA genotype was determined in 61% of the patient group and in 94% of the control group, and it was statistically low in the patient group. These results suggest that codon 54 polymorphism in the MBL gene may play a role in susceptibility to bacterial meningitis in children.

Key words: mannose binding lectin, bacterial meningitis, susceptibility, polymorphism.

Mannose binding lectin (MBL) is a calcium-dependent lectin that plays an important role in innate immunity by activating the complement pathway and phagocytosis¹. MBL activates complement via MBL-associated serine proteases (MASP). MBL is produced mainly in the liver by hepatocytes².

Mannose binding lectin binds, with multiple lectin domains, to the carbohydrate molecules expressed on the surface of many microbial organisms³. Human MBL gene is located at the locus 10q11.2-q21 and consists of four exons. Exon 1 encodes the signal peptide and cysteine-rich region. Both exon 1 and 2 encode the collagen-like glycine rich region⁴. Exon 3 encodes a neck region and exon 4 is a carbohydrate recognition region. These domains combine to form a basic subunit⁵. These homotrimeric subunits oligomerize to form larger structures. MBL consists of oligomers ranging from dimers to hexamers of the trimeric subunits².

Mannose binding lectin is a C-type lectin and its lectin activity depends on its calcium-dependent carbohydrate recognition domains⁶.

There are wide variations in serum MBL levels in humans due to genomic polymorphisms in the MBL gene. The single nucleotide polymorphisms [codon 52 (allele D), codon 54 (allele B), codon 57 (allele C), normal (allele A)] in exon 1 of the MBL gene disrupt the assembly of MBL trimers or accelerate the degradation of the protein. As a consequence, functional MBL decreases in the circulation and this causes predisposition to infections and autoimmune diseases⁴,⁷. There are also additional polymorphisms in the promoter region of the MBL gene which affect MBL concentrations less severely⁸.

In this study, we aimed to examine the presence of any association between codon 54 and codon 57 polymorphisms in the MBL gene variants and bacterial meningitis.

Material and Methods

Codon 54 and codon 57 polymorphisms in exon 1 of the MBL gene were investigated in 31 patients (mean age: 4 years 6 months, range: 1 month - 9 years) diagnosed as having purulent meningitis in the Department of
Pediatrics, Faculty of Medicine, Ege University and Dr. Behcet Uz Children’s Hospital, Izmir, Turkey. Fifty healthy subjects (mean age: 29 years, range: 21 - 38 years old) who had no previous history of central nervous system infection were included as the control group. The study was performed between January and May 2004. The diagnosis was established as a result of the findings detected in the cerebrospinal fluid (CSF). Four of 31 patients (13%) with bacterial meningitis has positive CSF cultures. *Streptococcus pneumoniae* (*S. pneumoniae*) (2 patients), *Neisseria meningitidis* (*N. meningitidis*) and *Staphylococcus aureus* (*S. aureus*) were the pathogens isolated in the CSF cultures.

DNA was extracted from blood samples by standard techniques. Exon 1 of MBL gene was amplified by polymerase chain reaction (PCR). The primer’s sequences were 5’-TAGGACAGAGGGCATGCTC-3’ and 5’-CAGGCAGTTTCCTCTGGAAGG-3’. 349 bp PCR product was digested with BanI and MboII for codon 54 and codon 57, respectively. BanI digestion was performed at 50°C for 60 minutes with 5 units of enzyme and MboII digestion was performed at 37°C for 90 minutes with 3.5 units of enzyme. The normal allele (allele A) was cut into two fragments with BanI, 260 bp and 89bp. The variant allele (allele B) remained uncut. MboII cleaved the variant allele (allele C) into 270 bp and 79 bp fragments. The fragments were visualized by electrophoresis on 2% agarose gel.

The data obtained were analyzed with the Statistical Package for Social Science (SPSS) chi-square test for qualitative variables. P<0.05 was considered to be significant.

**Results**

The DNA fragments of the patients and normal controls on agarose gel electrophoresis are shown in Figure 1.

Codon 57 polymorphism was not detected in any of the subjects from either group. MBL codon 54 genotype and allele frequencies are given in Table I. B allele frequency was significantly higher in the patient group (22%) compared to the control group (3%) (p=0.00).

Table I. Comparison of Mannose Binding Lectin (MBL) Variant Gene Frequencies Between Patients With Purulent Meningitis and Control Subjects

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Bacterial meningitis n (%)a</th>
<th>Healthy control n (%)b</th>
<th>Chi-square test X² p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Genotypes AA</strong></td>
<td>19 (61)</td>
<td>47 (94)</td>
<td>29.569 &lt;0.001</td>
</tr>
<tr>
<td>AB</td>
<td>10 (32)</td>
<td>3 (6)</td>
<td>9.792 0.02</td>
</tr>
<tr>
<td>BB</td>
<td>2 (7)</td>
<td>–</td>
<td>3.307 0.69</td>
</tr>
<tr>
<td><strong>Alleles</strong></td>
<td><strong>A</strong></td>
<td>48 (78)</td>
<td>97 (97)</td>
</tr>
<tr>
<td><strong>B</strong></td>
<td>14 (22)</td>
<td>3 (3)</td>
<td></td>
</tr>
</tbody>
</table>

AB/BB genotype frequencies were 39% and 6% in the patient and healthy control groups, respectively, and the difference was statistically significant. AA genotype was found in 61% and 94% of the patient and healthy control groups, respectively, and it was significantly lower in the patient group (p=0.000). Three of four culture-positive patients (with pathogens *N. meningitidis*, *S. aureus* and *S. pneumoniae*) had AB genotype and one patient with positive CSF culture for *S. pneumoniae* had AA genotype.

**Discussion**

Since the discovery of the importance of the MBL pathway in complement activation, there has been a rising interest in structural variants of MBL that are associated with decreased plasma concentrations of the protein and susceptibility to infections. Turner et al. indicated four distinct functions of MBL: activation of complement, promotion of opsonophagocytosis, modulation of inflammation, and promotion of apoptosis.

MBL protein concentrations decrease about 10 times in individuals with the heterozygote phenotype for MBL variants. In homozygous or compound heterozygous, no protein could be detected.

There are many articles published concerning decreased MBL levels and associated clinical diseases. Summerfield et al. reported that infections in childhood were associated with the possession of MBL variants. Roy et al. found that individuals with homozygote for MBL variants were more susceptible to invasive pneumococcal diseases. Viral hepatitis, otitis media, necrotizing pulmonary aspergillosis, malaria, and human immunodeficiency virus (HIV) diseases are some of the infectious diseases that have been shown to be associated with MBL insufficiency.

Hibberd et al. investigated the relationship between the variants of the MBL gene with susceptibility to meningococcal diseases. They found that the proportion of people carrying MBL variant alleles was higher in the patients with meningococcal disease than in the controls. Kuipers et al. presented a case of familial meningococcal disease due to deficiency in MBL.

Codon 57 polymorphism was not found in any of our patients nor the control group, supporting the previous studies related to the Turkish population. This polymorphism is mostly seen in the South African population. The frequency of codon 54 polymorphism (allele B) was significantly higher in the patients with purulent meningitis compared to the control group (Table I). AA genotype was found in 61% of the patient group versus in 94% of the healthy control group.

In conclusion, codon 54 polymorphism in the MBL gene may play a role in susceptibility to bacterial meningitis whereas the homozygous state for normal allele is protective.

**REFERENCES**


