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 phagocytosis[1]. MBL activates complement via MBL-associated serine proteases (MASP). MBL is produced mainly in the liver by hepatocytes[2].

Mannose binding lectin binds, with multiple lectin domains, to the carbohydrate molecules expressed on the surface of many microbial organisms[3]. 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 region[4]. Exon 3 encodes a neck region and exon 4 is a carbohydrate recognition region. These domains combine to form a basic subunit[5]. These homotrimeric subunits oligomerize to form larger structures. MBL consists of oligomers ranging from dimers to hexamers of the trimeric subunits[2].

Mannose binding lectin is a C-type lectin and its lectin activity depends on its calcium-dependent carbohydrate recognition domains[6].

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 diseases[4,7]. There are also additional polymorphisms in the promoter region of the MBL gene which affect MBL concentrations less severely[8].

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.

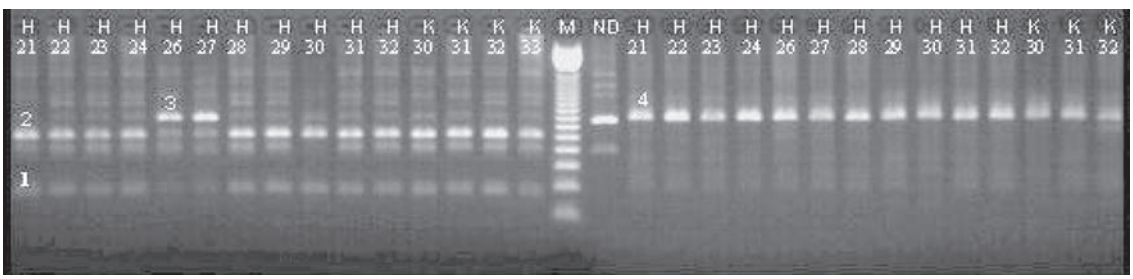
DNA was extracted from blood samples by standard techniques. Exon 1 of MBL gene was amplified by polymerase chain reaction (PCR). The primer's sequences were 5'-TAGGACAGAGGGCATGCTC-3' and 5'-CAGGCAGTTTCCTCTGGAAGG-3'. 349 bp PCR product was digested with BanI and MboII for codon 54 and codon 57, respectively. 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[4].

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%) ($p=0.00$).



H: Patient. K: Control. M: 50 bp DNA marker. ND: Non-digested PCR product.

Fig. 1. DNA fragments on agarose gel electrophoresis after restriction enzyme digestion of exon 1 of the MBL gene. On the left side of the marker, 349 bp PCR product was digested with BanI for codon 54 polymorphism. MboII digestion was visualized on the right side of the marker. The normal allele (allele A) is cut into two fragments with BanI, 89 bp (1) and 260 bp (2). The variant allele (allele B) remains uncut (3). MboII cleaves variant allele (allele C) into 270 bp and 79 bp fragments. Normal allele (349 bp) remains nondigested (4).

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

	Bacterial meningitis n (%)[a]	Healthy control n (%)[b]	Chi-square test X^2 p
Genotypes AA	19 (61)	47 (94)	29.569 <0.001
AB	10 (32)	3 (6)	9.792 0.02
BB	2 (7)	–	3.307 0.69
Alleles A	48 (78)	97 (97)	15.622 <0.001
B	14 (22)	3 (3)	

[a]n:31, [b]n:50.

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[9-11]. Turner et al.[4] 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[7].

There are many articles published concerning decreased MBL levels and associated clinical diseases. Summerfield et al.[12] reported that infections in childhood were associated with the possession of MBL variants. Roy et al.[13] 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[14-18].

Hibberd et al.[9] 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.[19] 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[8]. This polymorphism is mostly seen in the South African population[20]. 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

1. Terai I, Kobayashi K, Matsushita M, Miyakawa H, Mafune N, Kikuta H. Relationship between gene polymorphisms of mannose-binding lectin (MBL) and two molecular forms of MBL. Eur J Immunol 2003; 33: 2755-2763.
2. Dumestre-Perard C, Ponard D, Arlaud GJ, Monnier N, Sim RB, Colomb MG. Evaluation and clinical interest of mannan binding lectin function in human plasma. Mol Immunol 2002; 39: 465-473.
3. Song le H, Binh VQ, Duy DN, et al. Mannose-binding lectin gene polymorphisms and hepatitis B virus infection in Vietnamese patients. Mutat Res 2003; 522: 119-125.
4. Turner MW. The role of mannose-binding lectin in health and disease. Mol Immunol 2003; 40: 423-429.
5. Lipscombe RJ, Sumiya M, Hill AV, et al. High frequencies in African and non-African populations of independent mutations in the mannose binding protein gene. Hum Mol Genet 1992; 1: 709-715.
6. Kilpatrick DC. Mannan-binding lectin and its role in innate immunity. Transfus Med 2002; 12: 335-352.
7. Garred P, Larsen F, Madsen HO, Koch C. Mannose-binding lectin deficiency--revisited. Mol Immunol 2003; 40: 73-84.
8. Madsen HO, Garred P, Thiel S, et al. Interplay between promoter and structural gene variants control basal serum level of mannan-binding protein. J Immunol 1995; 155: 3013-3020.
9. Hibberd ML, Sumiya M, Summerfield JA, Booy R, Levin M. Association of variants of the gene for mannose-binding lectin with susceptibility to meningococcal disease. Lancet 1999; 353: 1049-1053.
10. Lipscombe RJ, Sumiya M, Summerfield JA, Turner MW. Distinct physicochemical characteristics of human mannose binding protein expressed by individuals of differing genotype. Immunology 1995; 85: 660-667.
11. Super M, Thiel S, Lu J, Levinsky RJ, Turner MW. Association of low levels of mannan-binding protein with a common defect of opsonisation. Lancet 1989; 2: 1236-1239.
12. Summerfield JA, Sumiya M, Levin M, Turner MW. Association of mutations in mannose binding protein gene with childhood infection in consecutive hospital series. Br Med J 1997; 314: 1229-1232.

13. Roy S, Knox K, Segal S, et al. MBL genotype and risk of invasive pneumococcal disease: a case-control study. Lancet 2002; 359: 1569-1573.

14. Thomas HC, Foster GR, Sumiya M, et al. Mutation of gene of mannose-binding protein associated with chronic hepatitis B viral infection. Lancet 1996; 348: 1417-1419.

15. Richardson VF, Larcher VF, Price JF. A common congenital immunodeficiency predisposing to infection and atopy in infancy. Arch Dis Child 1983; 58: 799-802.

16. Crosdale DJ, Poulton KV, Ollier WE, Thomson W, Denning DW. Mannose-binding lectin gene polymorphisms as a susceptibility factor for chronic necrotizing pulmonary aspergillosis. J Infect Dis 2001; 184: 653-656. Epub 2001 Jul 24.

17. Luty AJ, Kun JF, Kremsner PG. Mannose-binding lectin plasma levels and gene polymorphisms in Plasmodium falciparum malaria. J Infect Dis 1998; 178: 1221-1224.

18. Nielsen SL, Andersen PL, Koch C, Jensenius JC, Thiel S. The level of the serum opsonin, mannan-binding protein in HIV-1 antibody-positive patients. Clin Exp Immunol 1995; 100: 219-222.

19. Kuipers S, Aerts PC, Cluysenaer OJ, et al. A case of familial meningococcal disease due to deficiency in mannose-binding lectin (MBL). Adv Exp Med Biol 2003; 531: 351-355.

20. Hoal-Van Helden EG, Epstein J, Victor TC, et al. Mannose-binding protein B allele confers protection against tuberculosis meningitis. Pediatr Res 1999; 45: 459-464.