The individual course of hepatitis B virus (HBV) infection depends on three factors: The route of transmission, virulence of the virus, and immune system of the host. Innate and adaptive immunity participate in the pathogenesis of HBV infection. During the acute phase of the disease, 90% of the virus is destroyed by the innate immune system. Mannose-binding lectin (MBL) is a member of innate immunity that activates complement system through lectin pathway. MBL deficiency is associated with susceptibility to infectious diseases. In this study, the relation between MBL gene polymorphism and chronic hepatitis B infection in children is evaluated.

**Patients and Methods:** The study included 67 children with chronic hepatitis B and 99 healthy controls. The hepatitis B patients were divided into immuntolerant, chronic inactive, and treatment groups according to their laboratory findings. MBL gene codon 52, 54, and 57 polymorphisms were studied with polymerase chain reaction in all patients and controls. The associations of MBL gene polymorphism with clinical, laboratory, and histopathologic findings were evaluated.

**Results:** Homozygous codon 54 polymorphism of MBL was found significantly higher in chronic hepatitis B patients than controls. Rate of the polymorphism was similar in all groups and, responsive and nonresponsive patients in the treatment group.

**Conclusions:** The hepatitis B patients who are homozygous for codon 54 of MBL are prone to develop chronic infection. Longitudinal studies with larger groups are needed.

**Key Words:** Childhood, chronic hepatitis B, innate immunity, mannose-binding lectin

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pathogen. As a result, MBL activates complement on HBsAg–MBL complexes through the lectin complement pathway contributing to the viral clearance.\[6\]

Increased levels of immune complexes are associated with inflammatory damage in chronic liver disease. MBL-mediated complement activation could provide immune complex removal during HBV infection. Therefore, low MBL levels may lead to defective complement activation, poor clearance of immune complexes with subsequent deposition in the liver, and inflammation.\[7\] The other possible mechanism about the regulation of inflammatory cytokines by MBL is the association of low MBL levels with increased production of inflammatory cytokines, such as interleukin-6 (IL-6), IL-1-beta, and tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)) by monocytes. Enhanced production of IL-6, a fibrogenic cytokine, can activate stellate cells to contribute to fibrogenesis and further cirrhosis.\[8\] Consequently, MBL seems to have a role against inflammation and fibrogenesis in the liver tissue during the course of chronic hepatitis B (CHB) infection.

The recombinant MBL for therapeutic use is not available currently but phase studies are going on. It is considered to be used in MBL-deficient individuals with recurrent infections and for acute or chronic infections, where MBL deficiency worsens the course of the disease.\[9\]

The aim of this study is to evaluate the relationship between MBL gene polymorphisms and CHB infection. This study is the first to investigate the association between CHB and MBL alleles in children.

PATIENTS AND METHODS

The study included 67 children between 2 and 18 years of age with the diagnosis of CHB and 99 hepatitis B infection-negative healthy controls. The CHB group was divided into three groups according to the disease activity. Group 1 included immuntolerant patients (n: 16, 23.8\%), Group 2 included spontaneously seroconverted chronic inactive patients (n: 23, 34.3\%), and Group 3 included patients who received treatment for chronic active hepatitis (n: 28, 41.7\%). The treatment given was interferon (IFN)-\(\alpha\)-2b 5 MU/m\(^2\), three times-a-week for 24 weeks, continued with lamivudine (LAM) 3 mg/kg/day, for 36 months. The treatment group was also divided into two according to the therapy response. Seventeen patients were responders (60\%) and 11 patients were nonresponders (40\%). The definitions of groups are shown in Table 1.

The ethical committee of Uludag University approved the study and written informed consent was obtained from the parents. Patients with acute/chronic diseases other than chronic hepatitis B were excluded. Age, gender, and follow-up period of all patients were recorded and for the treatment group, medications, duration of therapy, and responses were documented. If done, liver biopsy reports of patients in groups 2 and 3 were collected. Three milliliters of blood with ethylenediaminetetraacetic acid and 5 mL sera were collected from all and were kept at \(-20^\circ\text{C}\). Due to the influence of HBV genotype on the course of chronic infection, virus genotyping was performed with a simple polymerase chain reaction (PCR) method defined by Eroğlu et al.\[11\] Genotypes were classified as D and non-D due to the high percentage of D genotype in Turkey.

MBL Genotyping

DNA was extracted from blood samples using standard techniques. Codons 54, 57, and 52 polymorphisms in the exon 1 of the MBL2 gene were genotyped in all patients in the study group using the PCR and sequence-specific primers. The primer sequences were 5′-TAGGACAGAGGGCATGCTC-3′ and 5′-CAGGCAGTTTCCTCTGGAAGG-3′. The PCR product (349 bp) was digested with two restriction enzymes: BanI and MBOII. The wild allele (Allele A) was cut into two fragments with BanI. The variant allele B remained uncut; MBOII cut variant allele C into two fragments and the variant allele D was cut into two fragments with both BanI and MBOII. Products were projected through electrophoresis on 2% agarose gel.[12,13]

The statistical analysis was done by using SPSS 16.0™. The frequencies of categorical variables were analyzed with Chi-square test and Shapiro–Wilk test was used for the assessment of the distribution of data. The difference between two variables with abnormal distribution was analyzed with Mann–Whitney \(U\) test. \(P < 0.05\) was considered as significant. The power analysis of the study was done, and we found out the value (1–\(\beta\)) to be 89\%.

RESULTS

MBL gene polymorphism was analyzed in 67 CHB patients (37 M, 50 F) and 99 healthy controls (52 M, 47 F). The mean age of children was 13.8 \(\pm\) 4.1 years and the mean follow-up period of CHB group was 4.09 \(\pm\) 3.64 years. Sixty-seven percent of mothers and 31\% of fathers were infected with hepatitis B virus. However, there was no clear data about the route of transmission. General characteristics of the groups are shown in Table 2.

Hepatitis B virus genotyping was done in 34 patients on serum samples and in five patients on liver biopsy specimens. Genotype D was found in 89.7\% (n = 35) and non-D in 10.3\% (n = 4). The distribution of non-D genotype among groups was two in immuntolerant group, one in therapy-responsive group, and one in nonresponsive group. The incidence of the patients infected with non-D genotype did not show any statistical difference in groups (two patients
Table 1: Definitions of the groups

| Group   | Definition               | Characteristics                                                                 |
|---------|--------------------------|---------------------------------------------------------------------------------|
| Group 1 | Immuntolerant            | HBsAg(+), HBeAg (+) Normal ALT, HBV DNA (+) Liver biopsy not needed             |
| Group 2 | Chronic inactive         | HbsAg(+), Anti HBe (+) (spontaneous) Normal ALT HBV DNA undetectable Liver biopsy not needed |
| Group 3 | Treatment group          | HbsAg (+), HBeAg (+), HBV DNA >10⁵ copy/ml, ALT >2x UNL Knodell index >4 on liver biopsy** |

IFNa-2β 5MU/m², three times-a-week for 24 weeks continued with Lamivudine 3 mg/kg/day, for 36 months

Responder
Anti HBe (+) (during/after treatment)
Loss of HBV DNA
ALT normalization

Non-responder
No change in biochemical or virological markers

Table 2: General characteristics of groups

|                          | Group 1 | Group 2 | Group 3 | P  |
|--------------------------|---------|---------|---------|----|
| Gender (M: F)            | 9:7     | 13:10   | 15:13   | NS |
| Age (year) mean±SD       | 11.69±4.45 | 14.65±3.42 | 14.32±4.23 | NS |
| Follow up period (year)  | 4.44±4.14 | 2.80±3.16 | 4.97±3.62 | NS |
| HBV genotype D (%)       | 88.2    | N/A     | 92.8    | NS |

M: Male, F: Female, SD: Standard deviation, HBV: Hepatitis B virus

in Group 1, and one patient each in the other groups, \( P = 0.23 \); however, the numbers are limited.

The distributions of MBL codon 54 allele in the study group and the controls were in Hardy–Weinberg equilibrium. All the four patients who were infected with non-D HBV genotype had wildtype MBL genotype (A/A). Codon 57 and 52 polymorphisms were not found in any of the subjects. The homozygous codon 54 polymorphism (BB genotype) was found significantly higher in CHB patients than the control group (8.9% vs 1.01%, \( P = 0.004 \)); however, heterozygous B allele (AB genotype) was significantly higher in the control group (11.95% vs 27.29%, \( P = 0.005 \)) [Table 3].

The frequencies of codon 54 polymorphism were similar in all CHB subgroups \( P = 0.75 \). The presence of MBL codon 54 polymorphism did not have any influence on therapy response \( P = 0.79 \) [Figure 1].

Liver histopathology reports of 25 patients in Group 2 (done during the active hepatitis phase) and Group 3 (before the treatment) could be obtained. Twenty-one patients had wildtype MBL2 gene, whereas four patients had homozygous codon 54 polymorphism. The results revealed that the patients with homozygous codon 54 polymorphism had higher Knodell index than the wild ones but without a statistical significance [Table 4].

**DISCUSSION**

MBL is a calcium-dependent C-type lectin and participates in innate immunity by complement activation. MBL gene has three defined polymorphisms that impair the function of MBL and decrease serum levels. Polymorphisms in codons 54, 57, and 52 are named as alleles B, C, and D, respectively. Prevalence of polymorphisms varies widely according to ethnic origin. B allele is found in 80% of healthy population in South Africa, and in 25% of whites. C allele is rarely found in Europeans, whereas its frequency is approximately 50% in Africans. Homozygous and heterozygous B allele frequencies in healthy Turkish population are 2%–6% and 12%–20%, respectively. In our study, we found homozygous B allele in 8.9% of children with chronic hepatitis B, which is significantly higher than the healthy population. However, the rate of heterozygous codon 54 polymorphism (AB genotype) was significantly higher in the control group, without any clinical consequence.
Figure 1: The comparison of MBL genotypes in CHB patients

Table 3: MBL genotypes in CHB patients and controls

| Genotype | CHB patients (n=67) | Controls (n=99) | P     |
|----------|---------------------|----------------|-------|
| AA       | 53 (79.24)          | 71 (71.70)     | >0.05 |
| AB       | 8 (11.93)           | 27 (27.29)     | 0.005 |
| BB       | 6 (8.94)            | 1 (1.01)       | 0.004 |

MBL: Mannose-binding lectin, CHB: Chronic hepatitis B

Table 4: The association of MBL genotype with liver histopathology in CHB

| Liver histology scoring (Knodell) | Genotype | P   |
|----------------------------------|----------|-----|
|                                  | AA (n=21) | BB (n=4) |
| Knodell index                     | 7.19±3.80 | 11.00±0.81 | 0.062 |
| Necrosis                          | 3.85±2.63 | 6.75±0.95  | 0.041 |
| Inflammation                      | 2.48±1.28 | 4.00±0.81  | 0.034 |
| Fibrosis                          | 1.19±1.07 | 1.0±0.0    | 0.73  |

MBL: Mannose-binding lectin, CHB: Chronic hepatitis B

The association of MBL polymorphism on the progression of CHB is presented in a limited number of studies and only in adult patients. Recently, Yuen et al.\[^{17}\] emphasized the importance of allele B on the persistence and progression of HBV infection. Thio et al.\[^{18}\] studied the natural course of hepatitis B infection in 527 patients in the United States and their results revealed that while the patients with allele B developed persistent disease, the ones without MBL polymorphism recovered spontaneously. In 2010, Filho et al.\[^{19}\] investigated the association of MBL2 polymorphism with susceptibility to hepatitis B infection, and their results showed a protective effect of MBL against hepatitis B infection. However, Hang-di et al.\[^{20}\] published a meta-analysis including 17 adult studies, 10 of which were methodically similar to our study. The results of the meta-analysis showed no association between MBL2 gene polymorphisms and susceptibility of CHB. Our study presents the first pediatric data on this subject. We found that the children with CHB had homozygous codon 54 polymorphism in significantly higher percentages than healthy controls, and this result reveals that the children with homozygous MBL codon 54 polymorphism are prone to develop chronic infection when they are infected with hepatitis B virus.

MBL has inhibitory effects on the release of proinflammatory cytokines resulting in a decreased inflammatory liver damage.\[^{6,18}\] In the present study, it has been observed that homozygous codon 54 polymorphism of MBL is associated with a higher Knodell index including inflammation and necrosis scores, without any statistical significance. This observation may be an early indicator of a significant liver damage, which will be evident in adulthood.

As mentioned, MBL binds to surface carbohydrate structures of various microorganisms. Hepatitis C virus (HCV) bears sugar moiety, such as glycoprotein E1 and E2, on its surface as HBV does, so it is not surprising that biological behavior of MBL has similar influence on elimination of HCV from the circulation of infected host.\[^{21}\] The role of MBL gene polymorphism on the progression of chronic HCV was investigated previously in a limited number of studies. In the year 2000, Sasaki et al. investigated the association of MBL polymorphism and chronic HCV infection, and they found that the patients with codon 54 mutation were at a higher risk to develop chronic active hepatitis and cirrhosis.\[^{22}\] Koutsounaki et al. studied the relationship between MBL
gene polymorphism and the outcome of HCV-infected patients and they found that the gene polymorphism was associated with progressive liver inflammation and cirrhosis.\(^{[21]}\) Another study from Brazil suggested that allele B was associated with progressive HCV infection and found that to be more frequent in the ones with cirrhosis than in the ones without.\(^{[24]}\) Similarly, we found a higher Knodell index (without statistical significance) in the ones with B allele than in the ones without. In another study from Brazil, 111 chronic HCV patients were searched for MBL gene polymorphisms and the results were similar to our study that mutant allele frequency was higher in the infected group than in the healthy controls. The authors concluded that MBL might represent an important antiviral role in the first stages of HCV infection.\(^{[23]}\) The effect of MBL gene polymorphism on the therapy response in chronic HCV patients has been studied in 1998 by Matsushita et al.\(^{[21]}\) Although the frequency of codon 54 was not different in HCV-infected patients and controls, the presence of codon 54 polymorphism was found in association with decreased response to IFN therapy in chronic hepatitis C patients. In our study, 28 of 67 patients (41.7%) were treated with IFN ± LAM and none of the patients were LAM resistant. Sustained HBe seroconversion was achieved in 60%. As a result, MBL gene polymorphism did not have any influence on the therapy response in our CHB patients.

**CONCLUSION**

The higher incidence of homozygous codon 54 polymorphism in our CHB patients than the healthy controls indicates the importance of MBL2 gene polymorphism in the persistence of HBV infection in children. Further studies including longitudinal follow-up of children until adulthood are needed to clarify the association of MBL gene polymorphism with long-term complications of chronic hepatitis B.

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Authors’ contributions:
- Gulin Erdemir is assistant in the project, data collection, laboratory work, interpretation of results
- Tanju Ozkan is the project coordinator, interpretation of results with an aspect of pediatric gastroenterologist
- Taner Ozgur, Hese Cosar, assistant in the project, data collection, statistical analysis, interpretation of results
- Ferah Budak, Huseyin Onay, assistant in the project, laboratory work, interpretation of laboratory results
- Sara Sebem Kilic, assistant in the project, interpretation of results with an aspect of pediatric immunologist.

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