Mannose-binding lectin gene polymorphisms are not associated with susceptibility to hepatitis C virus infection in the Brazilian Amazon region

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ABSTRACT

The present study compares the genotype frequencies between two population groups composed by 73 hepatitis C virus (HCV)-infected patients and 92 seronegative controls and investigates the role of allele variants as a possible factor in the susceptibility to HCV infection and the influence on disease progression.

The identification of MBL* B and MBL* C alleles was performed by restriction fragment length polymorphism analysis of the 349-bp product using Ban I and Mbo I restriction enzymes, respectively, and a polymerase chain reaction–sequence-specific polymorphism for discrimination of MBL*D. The analysis of allele and genotype frequencies between an HCV-infected group and seronegative controls did not indicate significant differences. The comparison of chronically infected subjects with and without liver cirrhosis was also not statistically significant. The odds ratio estimations were not significant, and the values obtained cannot suggest that the presence of allele variant MBL* B could have some influence in the risk of HCV infection progression to liver cirrhosis and that the presence of allele MBL*D could confer some protection against disease progression, but a larger sample size is necessary to confirm the present results.

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1. Introduction

Hepatitis C virus (HCV), a member of the Flaviviridae family, is considered a global public health problem and approximately 170 million individuals around the world are currently infected [1–3]. HCV infection usually evolves to chronic hepatitis and may progress to liver cirrhosis and hepatocellular carcinoma [4]. Furthermore, the natural history and outcome of HCV infections are usually associated with the interaction of virus and the host immune response [5].

Mannose-binding lectin (MBL) is a liver-derived pluripotent serum lectin that has a role in the host innate immune system [6]; it binds with high affinity to mannose or other carbohydrate components of viruses, bacteria, and yeasts [7]. MBL function is directly associated with its serum concentrations, which are determined by the interplay between promoter and structural gene mutations [8,9].

Three mutations of the wild allele MBL*A have been described in the structural region of the molecule (codons 52, 54, and 57) and three allelic variants named MBL*D, MBL*B, and MBL*C, respectively [10]. The occurrence of these variants has been associated with MBL serum deficiency and, consequently, variations in susceptibility/resistance to infection by various pathogens [7,11–16].

Recently, it has been suggested that MBL gene polymorphisms can influence the course of viral hepatitis caused by HCV, as well as the response to treatment [17,18]. Thus, the present study aims to compare the genotype and allele frequencies between HCV-infected patients and seronegative controls and investigates the role of the polymorphisms in susceptibility to HCV infection and the progression to chronic hepatitis.

2. Subjects and methods

2.1. HCV-infected patients

The group comprised a total of 73 blood sample donors (33 females and 40 males, 15 to 70 years old) residing in Belém (the capital of the state of Pará, Brazil), who tested positive for anti-HCV and HCV-RNA (real-time polymerase chain reaction [PCR] and PCR qualitative). A total of 35 HCV chronically infected patients were included in the present group. Sample collections occurred from August 2004 to December 2005 and were obtained from the Mo-
Polymorphism as described by Steffensen was detected following a polymerase chain reaction–single-strand equilibrium, were tested through electrophoresis, as previously described [8]. The presence of the product using performed by restriction fragment length polymorphism analysis.

The identification of the MBL A, MBL B, and MBL C alleles was performed by restriction fragment length polymorphism analysis of the product using BanI and MboII, followed by a 2% agarose gel electrophoresis, as previously described [8]. The MBL D allele was detected following a polymerase chain reaction–single-strand polymorphism as described by Steffensen et al. [19].

### Table 1

**Distribution of allele frequencies among HCV-infected and seronegative controls**

<table>
<thead>
<tr>
<th>Alleles</th>
<th>HCV-infected n (%)</th>
<th>Controls n (%)</th>
<th>χ²</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>MBL A</td>
<td>101 (69.18)</td>
<td>122 (66.30)</td>
<td>0.307</td>
<td>0.6632</td>
</tr>
<tr>
<td>MBL O</td>
<td>45 (30.82)</td>
<td>62 (33.70)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MBL B</td>
<td>18 (12.33)</td>
<td>28 (15.22)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MBL C</td>
<td>10 (6.85)</td>
<td>6 (3.26)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MBL D</td>
<td>17 (11.64)</td>
<td>28 (15.22)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>146 (100)</td>
<td>184 (100)</td>
<td>3.511</td>
<td>0.3193</td>
</tr>
</tbody>
</table>

n = number of chromosomes sampled. MBL*O represents the summation of variant alleles (MBL B, MBL C, and MBL D).

### Table 2

**Distribution of genotype frequencies among HCV-infected patients and seronegative controls**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>HCV patients n (%)</th>
<th>Controls n (%)</th>
<th>χ²</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homozygous AA</td>
<td>35 (47.94)</td>
<td>42 (45.65)</td>
<td>0.086</td>
<td>0.8917</td>
</tr>
<tr>
<td>Heterozygous AO</td>
<td>31 (42.46)</td>
<td>38 (41.31)</td>
<td>0.023</td>
<td>0.9931</td>
</tr>
<tr>
<td>AB</td>
<td>11 (15.07)</td>
<td>19 (20.65)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>AC</td>
<td>05 (5.32)</td>
<td>04 (4.35)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>AD</td>
<td>15 (20.55)</td>
<td>15 (16.30)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Homozygous OO</td>
<td>07 (9.60)</td>
<td>12 (13.04)</td>
<td>0.477</td>
<td>0.6564</td>
</tr>
<tr>
<td>BB</td>
<td>01 (1.37)</td>
<td>01 (1.09)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BC</td>
<td>04 (5.48)</td>
<td>01 (1.09)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BD</td>
<td>01 (1.37)</td>
<td>06 (6.52)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CD</td>
<td>01 (1.37)</td>
<td>01 (1.09)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>DD</td>
<td>00 (0.00)</td>
<td>03 (3.26)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>73 (100)</td>
<td>92 (100)</td>
<td>0.481</td>
<td>0.7863</td>
</tr>
</tbody>
</table>

n = number of individuals sampled.

### 3. Results

MBL gene polymorphisms were investigated in two population groups and the genotype frequency distributions were in agreement with Hardy–Weinberg equilibrium. Alleles occurred with frequencies ranging from 3.26 to 69.18%, but the differences were not statistically significant (Table 1). Additionally, the analysis of OR to compare the risk between cases and controls regarding the presence of the alleles MBL A and MBL O indicated no association of the allele frequencies with HCV infection (OR = 0.8767; p = 0.6632; 0.5503 ≤ μ = 1.3967).

Genotype AA was the most frequent in both groups, with frequencies ranging from 45.65% in the controls to 47.94% in the HCV-infected group. Genotypes BC, DD, and BD were observed in both groups, but genotype CC was not detected (Table 2). Furthermore, the combined analysis of A/O and O/O genotypes (Table 2), as well as the allele MBL O (Table 1), indicated no significant differences.

When genotypes were grouped, the heterozygous AO occurred with frequencies of 42.46% among HCV-infected patients and 41.31% among the controls. Despite the finding of homozygous OO in a slightly higher frequency among the control group, there was no statistically significant difference detected (Table 2).

Clinical information was obtained from 35 chronically infected HCV patients with (14) and without (21) liver cirrhosis. According to the allele distribution, the variant MBL B was the most frequent (14.29%) among patients with hepatic cirrhosis and the allele MBL D was the most frequent among patients who did not present a clinical diagnosis of cirrhosis (11.90%), but the differences were not statistically significant (Table 3).

Genotype AB was more frequently seen among patients who developed hepatic cirrhosis (28.57%) than among patients who did not (14.29%; data not shown). The genotype AD demonstrated a higher frequency among patients with chronic hepatitis without (23.81%) compared with patients with cirrhosis (7.14%), but the differences were not statistically significant.

### 4. Discussion

The present study investigated the occurrence of polymorphisms of the MBL gene and its possible role as a risk factor for the chronicity and progression to cirrhosis among HCV-infected subjects.

Polymorphisms of MBL are frequently associated with higher frequencies of viral infections [6,10,22]. In particular, the allele MBL B is usually found more frequently among HIV-1-infected persons [12,23–26], but no association of that allele was reported regarding HCV infections.

A previous report describing polymorphism among HCV-infected patients from the northeastern region of Brazil indicated the

2

\[ \text{OR} = \frac{\text{p}_{\text{cases}}}{\text{p}_{\text{controls}}} \]

...
role of the allele MBL*C in susceptibility to the infection because this mutation was detected in a higher frequency than in controls [27]. In the present paper, although the allele was described in a higher frequency in the group examined, it was not statistically significant. It is noteworthy that the northeast and the northern geographic areas of Brazil experienced different genetic contributions during their colonization processes [28]. The two population groups exhibit different compositions of Caucasians (34% in the northeast vs. 47% in the Amazon region of Brazil), Indians (22% vs. 41%), and Africans (44% vs. 22%). It is possible that such differences may account for the different results described. However, it must be noted that the small differences observed in the present study concerning the genotype frequencies could not be attributed to ethnic background because both HCV-infected and control groups were originated and selected from the same population residing in Belem, the capital of Pará State.

Allele MBL*D is commonly associated with uninfected control subjects [11,12,23], as seen here with HCV. The plasma concentration of MBL does not exceed normal reference values among MBL*D allele carriers, suggesting a distinct effect from that of alleles MBL*B and MBL*C [8]. It is possible that the protein is partially incorporated as a stable oligomeric structure that is not seen in proteins from variants MBL*B and MBL*C [29]. Although this observation could explain the normal serum levels of MBL among AD heterozygous subjects, its protective action must be further exploited.

The numbers herein described for the frequencies of genotypes BB and OO were low and were present in both infected and seronegative groups with no significant differences, a situation different from what has been described as a positive influence for the increase in susceptibility to virus infections [11,12,23,27,30]. It is important to keep increasing the number of subjects examined to confirm previous descriptions of association between MBL polymorphisms and susceptibility to HCV infections [17,27,31–33], which was not found in the present paper.

Chronic infections of HCV commonly evolve to liver cirrhosis and hepatic cancer [4] and there is some information that associates the polymorphism of the MBL gene with the progression of disease to a poorer prognosis, as well as to the therapeutic lack of response [27,32]. MBL*B was not associated with the progression of HCV infection to a chronic infection and to liver cirrhosis [17]. The results obtained in the present paper indicate the allele is present in a higher frequency than in cirrhosis patients compared with what was recorded for chronic carriers. Although the data may indicate a possible association of the allele as a marker for the poorer progression, it was not possible to ascertain statistical significance as reported for hepatitis B virus infection [34].

The genetic information described among the present group of HCV-infected subjects highlights the possible influence of the polymorphisms of the MBL gene and the outcome of the infection, as previously reported [33]. The present study design requires the involvement of a greater number of subjects to yield verifiable results of a possible association of the allele MBL*B with hepatic cirrhosis caused by HCV infection.

### Table 3

Distribution of allele frequencies among HCV chronically infected patients and a clinical diagnosis of liver cirrhosis

<table>
<thead>
<tr>
<th>Alleles</th>
<th>Chronic hepatitis With liver cirrhosis n (%)</th>
<th>Chronic hepatitis Without liver cirrhosis n (%)</th>
<th>OR</th>
<th>CI 95%</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>MBL*A</td>
<td>22 (78.57)</td>
<td>33 (78.57)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MBL*B</td>
<td>4 (14.29)</td>
<td>1 (3.57)</td>
<td>2.1667</td>
<td>0.4458 ≤ IC ≤ 10.529</td>
<td>0.5692</td>
</tr>
<tr>
<td>MBL*C</td>
<td>1 (3.57)</td>
<td>1 (2.38)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MBL*D</td>
<td>1 (3.57)</td>
<td>5 (11.90)</td>
<td>0.2741</td>
<td>0.0303 ≤ IC ≤ 2.4825</td>
<td>0.4328</td>
</tr>
<tr>
<td>Total</td>
<td>28 (100)</td>
<td>42 (100)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

n = number of chromosomes sampled. χ² = 2.3; p = 0.5122.

Acknowledgments

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References


