Hemochromatosis Mutations in Iranians with Hepatitis B Virus Infection

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In this study, the frequencies of the common hemochromatosis gene mutations were assessed in 75 Iranian subjects with chronic hepatitis B infection. We found that the major C282Y mutation was significantly more frequent in subjects infected with hepatitis B virus (4%) than in 194 control subjects (0%, P = .02; Fisher's exact test).

Hepatitis B virus (HBV) infection can cause a broad spectrum of disease, ranging from asymptomatic inactive HBV carriage to acute hepatitis, chronic hepatitis, cirrhosis, and primary hepatocellular carcinoma. The reasons for this variability in the pattern and clinical outcome of HBV infection are still poorly understood.

The role of iron in modulating the course of viral hepatitis was first highlighted by Blumberg et al. [1]. They found that patients with higher levels of serum iron or ferritin were less likely to achieve spontaneous recovery after acute HBV infection. Elevations in serum ferritin level and serum iron saturation percentage were also noted in patients with chronic hepatitis C [2–4]. Recent studies have indicated that, among patients with chronic hepatitis C, there is an increased prevalence of the C2824 mutation, the major mutation of the hemochromatosis gene (HFE) associated with hereditary hemochromatosis [5–7]. Patients with the C282Y mutation also have had more-advanced hepatic fibrosis than patients without this mutation [5, 8]. Few, if any, previous studies have focused on the relationship between HFE mutations and the persistence or natural history of HBV infection. The aim of the current study was to determine whether there is any difference between the frequencies of HFE mutations in hepatitis B surface antigen (HBsAg)-positive patients from central Iran, compared with a suitable control group. In addition, we sought to learn whether recessively inherited mutations in the HFE gene had any role in the persistence and/or chronicity of HBV infection.

During January 2001–December 2002, all patients who presented to RCGLD (Tehran) and who were positive for HBsAg were invited to participate in this study. A total of 75 unrelated patients (of which 46 were men and 29 were women, with a mean age of 40 years) were identified, and all agreed to participate. We stratified these subjects into those with inactive HBV carriage (n = 18) and those with chronic hepatitis B (n = 57). An inactive carrier was defined as a subject who had been HBsAg-positive for ≥6 months without any indication of activity of disease, such as an elevated serum alanine aminotransferase level (ALT) or the presence of hepatitis B e antigen (HBeAg). Of these 18 subjects, 9 were assessed and found to be positive for HBeAb. We were not able to detect HBV DNA in serum from any of the subjects because, at the time this study was done, this assay was not available. Serum samples from these subjects are currently unavailable. Subjects who tested positive for HBsAg and had a serum ALT level of ≥2 times the upper limit of the normal range (normal range, 5–40 μ/L) for at least 6 months (n = 54) or an ALT level between 1.5 and 2 times the upper limit of the normal range and a liver biopsy specimen with at least grade 4 histological activity (according to Knodell scoring) (n = 3) were classified as having chronic hepatitis B. All subjects tested negative for serum antibodies against hepatitis C virus (by ELISA), and their serum ceruloplasmin and α-1 antitrypsin levels were normal. Results of serologic tests for markers of autoimmune hepatitis (anti-nuclear antibodies and smooth muscle antibodies) were negative or positive only at low titers (<1/80). None of the subjects infected with HBV had AIDS. Testing for HIV antibodies was not performed. One hundred ninety-four asymptomatic control subjects without known diseases (of which 109 were men and 85 were women, with a mean age of 35 years) were recruited from patients of the primary care unit of the affiliated hospital of RCGLD and from the healthy staff of RCGLD. They had no history of liver disease, alcohol abuse, anemia, or iron over-
load. Control subjects tested negative for HBsAg, HCV antibodies, and HIV antibodies, and their serum levels of ALT, aspartate aminotransferase, and total bilirubin values were within normal ranges. Control subjects were not related to any of the subjects who tested positive for HBsAg; all subjects who subjects tested positive for HBsAg were natives of central Iran.

After obtaining informed consent, blood was drawn, and genomic DNA was extracted from whole blood. Presence of common mutations of the \textit{HFE} gene (C282Y and H63D) was assessed by PCR amplification of appropriate portions of the \textit{HFE} gene, followed by restriction fragment-length polymorphism (RFLP) analyses, as described elsewhere [9]. All of the non–wild-type results were confirmed by sequencing both DNA strands (Terminator Labeled Kit; Amersham), with consistent results. Data were analyzed using Stata software, version 8 (StataCorp). Differences in the proportions of mutations between patients and control subjects with mutations were assessed by the \( \chi^2 \) test or Fisher's exact test. Student's \( t \) test was used to test for a difference of mean serum ferritin levels between HBsAg-positive subjects and control subjects. The \( \alpha \)-error was set to \( \leq 0.05 \) for assessment of statistical significance.

For the major C282Y mutation, we found that 3 (4\%) of all HBsAg-positive subjects, but none of the control subjects, were heterozygous (\( P = .02, \) 2-tailed Fisher’s exact test). All 3 of these C282Y heterozygotes were in patients with chronic hepatitis B (frequency = 5.2\%), and there was a significant difference between these subjects and control subjects (\( P = .01, \) 2-tailed Fisher’s exact test). No statistically significant difference in the frequency of C282Y mutations was found between HBsAg-positive subjects and control subjects. The \( \alpha \)-error was set to \( \leq 0.05 \) for assessment of statistical significance. All subjects positive for HBsAg 75 3 (4\%) 10 (13.3\%) 0 (0.0\%) 0 (0.0\%)

The major finding of this study of \textit{HFE} mutations in Iranians with HBV infection is that the frequency of the major C282Y mutation in subjects who were HBsAg-positive was higher than in control subjects. Because of the lack of histological examination of hepatic examination, our ability to assess the contribution of a mutated \textit{HFE} to more-severe liver disease is limited. Although all patients with the C282Y mutation were in the group with elevated serum ALT levels, the difference in the frequency of the mutation was not significant, compared with that in the group with normal serum ALT levels, possibly because of the relatively small number of subjects in the latter group. Our findings are consistent with those of several recent reports that have focused on patients with chronic hepatitis C [5–7], but the findings of those reports contrast with that of Piperno et al. [10], who found no significant difference in the frequency of \textit{HFE} mutations between patients with chronic viral hepatitis (including 97 patients with chronic hepatitis C, 10 patients with chronic hepatitis B, and 3 patients that were positive for markers of both hepatitis B and C virus) and control subjects.

Although carriage of \textit{HFE} mutations is frequently observed in patients with hepatitis C, still unresolved is whether the influence of \textit{HFE} mutations on hepatitis C virus infection is solely due to the effects of iron levels, per se, or to other as yet poorly understood differences between subjects with and without the \textit{HFE} gene mutations. The product of the \textit{HFE} gene is a major histocompatibility complex type I protein, and several immunologic differences have been described between subjects with and without \textit{HFE} mutations [11–13], which may result in a different host-immune response in patients harboring \textit{HFE} mutations who also have viral hepatitis. However, the role of \textit{HFE} mutations in immunity is still undecided. In one recent study involving mice, targeted disruption of the \textit{HFE} gene was not associated with any obvious abnormalities in the numbers or subtypes of T cells [14].

**Table 1. Distribution of \textit{HFE} gene mutations in subjects positive for hepatitis B surface antigen (HBsAg) and in control subjects.**

<table>
<thead>
<tr>
<th>Study group</th>
<th>( n )</th>
<th>C282Y +/–</th>
<th>H63D +/–</th>
<th>C282Y +/+</th>
<th>H63D +/+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects with HBV carriage</td>
<td>18</td>
<td>0 (0.0%)</td>
<td>2 (11.1%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Subjects with chronic hepatitis B</td>
<td>57</td>
<td>3 (5.2%)</td>
<td>8 (14%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>All subjects positive for HBsAg</td>
<td>75</td>
<td>3 (4%)</td>
<td>10 (13.3%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Control subjects</td>
<td>194</td>
<td>0 (0.0%)</td>
<td>31 (16%)</td>
<td>4 (2.1%)</td>
<td></td>
</tr>
</tbody>
</table>

**Note.** HBV, hepatitis B virus; +, mutation was present; –, mutation was absent; +/–, subjects were heterozygous; +/+, subjects were homozygous.
Our results support the belief that the major HFE mutation C282Y increases the likelihood of persistence of viral infection and, perhaps, development of liver disease in persons infected with HBV. Of course, the study of larger numbers of HBV–infected patients and matched control subjects from Iran and other countries will be important for delineating the role of HFE mutations in hepatitis B virus infection more clearly.

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