HFE gene mutations, hepatic iron content, and histological severity in hepatitis C virus-induced chronic hepatitis

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ABSTRACT

Objective: to study whether any relationship exists between the C282Y and H63D mutations of the HFE gene, iron liver content, and the severity of histological damage in patients with hepatitis C virus (HCV)-induced chronic hepatitis.

Material and methods: in 72 patients diagnosed with HCV-chronic infection, naïve for antiviral therapy, and undergoing liver biopsy, the Knodell index was established, a morphometric evaluation of hepatic hemosiderin deposits was performed by using a semiautomatic method of image analysis, and mutations of the HFE gene were identified through a polymerase chain reaction on leukocyte genomic DNA by using specific restriction enzymes. The control group for the distribution of HFE genetic variants was composed of 181 healthy individuals with the same ethnic and geographical (white Spaniards) origin.

Results: (cases/controls): 1. Genotype distribution: a) mutation C282Y: no homozygotes, 6/23 heterozygotes, 66/158 without the mutation (not significant, n.s.); b) mutation H63D: 2/5 homozygotes, 26/52 heterozygotes, 44/124 without the mutation (n.s.). compound heterozygotes 2/6. 2. Allele frequencies: a) mutation C282Y: 0.042/0.064 (n.s.); b) mutation H63D: 0.208/0.171 (n.s.). Four C282Y heterozygous patients had stainable liver iron (p = 0.015 vs patients without mutations). Sixty-six patients were not carriers of the C282Y mutation; among them, 26.9% of 26 carriers and 15% of 40 non-carriers of the H63D mutation had liver stainable iron (n.s.). Knodell index score, gender, age at diagnosis, mode of transmission, and serum and liver iron values were not related to the HFE genotype.

Conclusions: our results suggest that the C282Y mutation, but not the H63D mutation, of the HFE gene is frequently associated with stainable iron in the liver in HCV-related chronic hepatitis. The HFE genotype is not related to the histological severity of the disease.

Key words: Hepatitis C virus. Chronic hepatitis C. HFE gene.

INTRODUCTION

Iron stored in tissues may cause damage because of its ability to generate free oxygen radicals that in turn may activate several inflammatory cascades through the release of proinflammatory cytokines, and modify structural or functional molecules of cells such as, for example, unsaturated fatty acids, proteins, and nucleic acids (1,2). These events are particularly relevant in the liver, an organ that normally contains apparent iron stores that are increased in various pathological circumstances. Hepatic iron content may be measured in a biopsy specimen by atomic absorption spectrometry, but if significant fibrosis or cirrhosis exist, or the amount of available tissue is very small, the margin of error is not negligible (3). Semi-quantitative methods (4) to evaluate the amount of iron present in histological preparations stained with iron-specific methods have been proposed (e.g., Perls' staining method).

Serum iron and ferritin levels are frequently elevated in chronic hepatitis C virus (HCV) infection (5). However, hepatic iron overload is less frequent and usually not very important (6), with a selective affinity for Kupffer cells (7). It has been suggested that the excess of hepatic iron may increase the risk of chronification of HCV infection (8) or accelerate the course of the disease (9).

Feder et al. (10) identified in 1996 two point mutations at the HFE gene. The Cys282Tyr (C282Y) mutation in homozygosis is responsible for more than 90% of genetic hemochromatosis cases in Caucasian populations. Heterozygotes for the C282Y allele frequently show higher
than normal iron serum indexes and liver iron content (11). The possible influence of the H63D mutation, both in homo- and heterozygosis, on liver iron content is a matter of controversy (12-14). Several groups have suggested that a possible relation may exist between these HFE gene mutations and the risk of several liver diseases, as is the case of alcoholic cirrhosis (15), non-alcoholic steatohepatitis (16), or porphyria cutanea tarda (17).

Data on the possible relationship between carriage of the HFE gene mutations, risk of severity of HCV-induced liver disease, and level of hepatic iron also have been controversial (18-30). The aims of this study have been to establish the frequency of HFE gene mutations in a group of white Spanish patients with chronic hepatitis C, and to detect whether these mutations are related to the degree of liver iron deposits and/or the severity of histological liver damage.

MATERIAL AND METHODS

All unrelated white Spanish patients diagnosed with chronic HCV infection and submitted for liver biopsy to the Gastroenterology Department of Hospital Clínico San Carlos between June 2000 and November 2001 were included. Epidemiological (age, sex, ethanol-consuming habits) and serum biochemical (serum aminotransferases, bilirubin, alkaline phosphatase, gammaglutamyltransferase, sideremia, transferrin saturation, and ferritin) data were recorded.

The control group was made up of unrelated healthy blood donors, all Caucasian Spaniards. Subjects whose samples were discarded for whatever reason were not included. Both patients and control subjects gave their informed consent to be included in the study.

For molecular analysis, genomic DNA was extracted from peripheral blood leukocytes (31). The study of the HFE gene was done by PCR (polymerase chain reaction) using the previously described oligonucleotides (10,32). For molecular analysis, genomic DNA was extracted from peripheral blood leukocytes (31). The study of the HFE gene was done by PCR (polymerase chain reaction) using the previously described oligonucleotides (10,32).

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Quantification of hemosiderin deposits in the liver was done on histological sections stained with Perls’ technique, by using a semiautomatic image analysis system (Olympus, Microimage version 4.0). Results are expressed in µm² of positive-blue stained areas by mm² of surface of hepatic lobe. The Knodell index (33) was established in all patients as a part of our standard study protocol.

The statistical analysis was performed by using tests for independent continuous variables (Student’s t test or Mann-Whitney’s U test, when appropriate) and the Chi square test (Mantel-Haenszel’s) for categorical variables; odds ratios and the 95% confidence intervals for dichotomical variables were calculated. The null hypothesis was rejected when p < 0.05 or the confidence interval did not include 1. The Rsigma (Horus Hardware) and EpInfo 6.2 (Center for Disease Control & Prevention, Atlanta, USA) statistical packages were used.

RESULTS

Seventy-two patients (40 males, mean age 45 years, S.S. 11.8, range 21-76) and 181 controls were included. Six patients (all male) admitted their drinking more than 50 g of ethanol daily. The control group was composed of 181 healthy blood donors.

The genotype distribution of the C282Y and H63D mutations is shown in table I, and the allelic distribution in table II. No homozygotes for the C282Y mutation were found, and no significant differences existed between cases and controls in the proportion of C282Y heterozygotes or C282Y allele frequency, both proportions being similar to those detected in other studies on the Spanish population (34). There were no differences, either, between allele frequencies and the three possible genotypes defined by the H63D mutation of the HFE gene. Two patients and six controls were compound heterozygotes C282Y/H63D.

Liver iron was detected in 17 patients (23.4%). The staining-index was low (less than 1,000 µm²/mm²) in three patients, intermediate (1,000-10,000 µm²/mm²) in eight patients, and high (more than 10,000 µm²/mm²) in six patients. Four of the six heterozygous patients for the C282Y mutation (one of them a compound heterozygote) had stainable iron deposits in the liver, which were intermediate in three and high in one (p vs patients without mutations: 0.015).

The 66 patients without the C282Y mutation were analyzed separately. Twenty-six were H63D carriers (24 heterozygotes and 2 homozygotes), and the remaining 40 were homozygous for the normal allele. Stainable liver

Table I. Distribution of HFE genotypes among 72 cases and 181 controls*

<table>
<thead>
<tr>
<th>HFE mutation</th>
<th>Group</th>
<th>No mutation No. (%)</th>
<th>Carriers No. (%)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Homozygotes/mixed heterozygotes/simple heterozygotes)</td>
<td></td>
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<tr>
<td>C282Y Cases</td>
<td>66 (92%)</td>
<td>6 (8%)</td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>158 (87%)</td>
<td>23 (13%)</td>
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</tr>
<tr>
<td>H63D Cases</td>
<td>44 (61%)</td>
<td>28 (39%)</td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>124 (68%)</td>
<td>57 (32%)</td>
<td></td>
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* No significant differences exist.
iron was detected in seven H63D carriers (26.9%) and six non-carriers (15%), (p = 0.19, not significant).

No significant differences were found when the four items of the Knodell index were separately analyzed in relation to the presence or absence of stainable iron in the liver (this analysis was performed in the whole series), or to carrier status for the H63D mutation (analysis restricted to the 66 patients without the C282Y mutation).

Gender, age at diagnosis, mode of HCV transmission, and serum and liver iron biochemical parameters were not related to the HFE genotype or the presence of liver stainable iron. Two of the six patients with a history of excessive ethanol intake had stainable iron in their livers; one (high iron level) was free of mutations, and the other (intermediate iron level) was a H63D heterozygote. Of the remaining 4 patients, two had no mutation, one was H63D homozygous, and the other H63D heterozygous. When these six cases were withdrawn from the analysis, results did not change in any significant manner.

**DISCUSSION**

The semi-quantitative morphometric method used in this study allowed to detect stainable iron stores in 23% of patients with chronic hepatitis C. This result is in agreement with previous data, indicating that hepatic iron overload is more frequent in these patients when compared to other chronic disorders of the liver (6). It should be stressed that the semi-quantitative analysis of liver iron stores based upon the visual assessment of iron-specific histological preparations is somewhat imprecise, and that low iron scores (1-2 points) may be detected in the normal liver (35). Therefore, we can only consider undoubtedly excessive scores higher than 2 (36). We only know a study that compared the gold standard of liver iron measures (atomic absorptiometry) with histological scoring methods, and the correlation found was rather weak (28). Nevertheless, the group that proposed a more elaborate histological index (with scores from 0 to 64 points) argued that their system was able to differentiate homozygotes from heterozygotes regarding the hemochromatosis mutation (4).

In our study we did not detect any significant differences in the frequencies of C282Y and H63D mutations of the HFE gene between cases and normal controls. This result is in agreement with most published reports (18-25, 28,29), although Pirisi et al. (26) detected a significant excess of both mutations in patients with HCV-positive chronic hepatitis, and Van Vlierberghe et al. (27) reached similar results in their study of the C282Y mutation.

Four of our six patients carrying the C282Y mutation (four simple heterozygotes and two mixed C282Y/H63D heterozygotes) had stainable iron in their liver biopsies. This finding agrees with some previously quoted studies (19,21,22,29,30), but other groups (18,20,25,27) failed to detect any relation between carrier status for the C282Y mutation and risk of hepatic iron overload.

We found a non-significant excess of H63D carriers among our patients with stainable iron in their liver biopsies. This finding is irrelevant in itself, but in accordance with reports of a higher iron content in the liver of patients with chronic hepatitis C who carry this mutation (19,21,22). Martinelli et al. (21) detected that the severity of inflammation and fibrosis in the liver was greater in this subgroup of patients, whereas Tung et al. (30) found that the extent of fibrosis was greater in patients with compensated HCV-related chronic liver disease who carried any one of both mutations versus non-carriers. In our study we found no differences in any of the four items of the Knodell index in relation with the presence or absence of these mutations.

Our results suggest that heterozygosis for the C282Y mutation may facilitate the accumulation of iron in the liver of patients with chronic hepatitis C, although the number of patients carrying this mutation in our series was very small. On the other hand, it may be concluded that the C282Y and H63D mutations of the HFE gene influence neither the risk of chronicisation of HCV infection nor the course of the disease.

**REFERENCES**


