Hemochromatosis Gene Mutations: Prevalence and Effects on Pegylated-Interferon and Ribavirin Therapy Response in Chronic Hepatitis C in Sardinia

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Background/Aims: **Considerable evidence suggests that iron could be a comorbid factor for liver injury in chronic hepatitis C (CHC). Elevated iron indices are frequently described in CHC and may impact negatively on the course of liver disease and on the response to interferon alfa therapy. The aim of this study was to evaluate the frequency of hemochromatosis gene mutations in Sardinian CHC patients, the association with iron overload and the impact on response to therapy.** ***Methods:*** **Sixty-nine CHC patients were enrolled. Iron indices, hepatic and viral parameters were detected. C282Y, H63D and S65C mutations were identified through a PCR. Liver biopsy was performed for hepatic fibrosis evaluation. All patients were treated for 6 months (viral genotype 2/3) or 12 months (viral genotype 1/4) with pegylated-interferon 180 mcg once weekly and ribavirin 1000–1200 mg/daily. Sustained virological response (SVR) was defined as undetectable HCV RNA 24 weeks after the end of treatment.** ***Results:*** **HFE gene mutation was detected in 29 patients (42%). The presence of HFE mutations was significantly associated with elevated transferrin saturation ($P < 0.01$). Hepatic fibrosis was more advanced in HFE mutation carriers (χ^2, $P = 0.04$). Among mutation carriers 27.5% achieved responses at the end of treatment compared with 60% of non-carriers ($P = 0.005$). Patients with HFE wildtype produced significant SVR compared with patients with HFE mutations ($P = 0.03$).** ***Conclusions:*** **The literature shows discordant results about the prevalence, hepatic distribution and possible therapeutic implications of iron overload in chronic hepatitis C. Our findings shows that HFE gene mutations could favor, synergically with CHC and other genetic or acquired factors, the development of liver damage and could influence the outcome of interferon treatment with higher rate of non-response.** **(J Clin Exp Hepatol 2012;2:211–217)**

Iron is an essential micronutrient which plays a key role in a wide range of biochemical pathways that govern cellular metabolism, including those that are essential for cellular respiration as well as DNA, RNA and protein synthesis. Iron balance is regulated at the absorptive step, but the mechanism by which the mucosa accomplishes this has not been defined. There is no effective physiological mechanism for the excretion of excess body iron, hence increased absorption of iron would increase body iron stores, mainly in the liver.[1] Iron has been shown to increase the formation of reactive oxygen intermediates that lead to lipid peroxidation and subsequent oxidative damage to proteins and nucleic acids.[2] Iron-induced oxidant stress is involved in this process as the primary cause of parenchymal cell necrosis or as activator of cells that are effectors or key mediators of hepatic fibrogenesis. The fibrogenic potential of iron in the liver is even more important when iron acts simultaneously with other hepatotoxic factors.[3]

The intestinal iron absorption appears to be disturbed in Hereditary Hemochromatosis (HH). The homozygous state in which both alleles of chromosome 6 possess the C282Y mutation or the compound heterozygous state with C282Y on one chromosome and H63D on the other, are the predominant genetic abnormalities associated with phenotypic HH.[4] A third mutation, S65C, is considered to be a rather new polymorphism.[5] "In Europe, the C282Y allele has a north to west frequency- decreasing gradient, with higher frequencies reported in Ireland (28.4%) and lower frequencies in Italy (3.2%). Conversely, the H63D allele has a higher frequency in southern Europe (Spain, 32.3%) and a lower frequency in the Celtic populations (5%).[6]

The Sardinian population is genetically differentiated from the other Caucasian populations. It represents a genetic isolate where the p.C282Y mutation is considered as rare or even absent. Candore et al studied the frequency of the HFE gene mutations in five Italian populations. In Italy, the allele frequency of the C282Y mutation decreases from

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Abbreviations: ALT: alanine aminotransferase; AST: aspartate aminotransferase; AP: alkaline phosphatase; CHC: Chronic hepatitis C; ETR: End of treatment response; GGT: g-glutamyl transpeptidase; HFE: Human hemochromatosis protein; HCV: Hepatitis virus C infection determination; HH: Hereditary Hemochromatosis; SVR: Sustained virologic response; TSI: Transferin saturation index; ULN: Upper normal limit; WT: wildtype
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northeast Italy (Friuli, 6%) to northwest Italy (Piemont, 4.8%) and to central Italy (Emilia-Romagna, 1.7%). However, this mutation is lacking in Sardinia. In contrast, no difference was observed in allele frequency of H63D in the five Italian regions (Friuli 12%—Sardinia 17.5%).[7] Several studies assessed that no association exist between the HFE genetic variants and chronic liver disease. Overall, only a few studies have suggested an increased prevalence of HFE mutations in CHC patients,[8,9] with respect to the general population; this observation was not confirmed in other studies.[10] Laboratory abnormalities of iron metabolism have been detected in 15–20% of heterozygotes, but heterozygosity for hemochromatosis is rarely associated with liver damage due only to iron overload. Complications have been recognized only when other disorders, such as porphyria cutanea tarda, chronic anemia, alcoholism and hepatitis are also present.[11] Over the last 20 years, considerable evidence suggested that a pathogenetic link exists between the iron content of the liver and viral hepatitis. Elevated iron indices are frequently described in CHC and may impact negatively on the course of liver disease and on the response to interferon alfa therapy.[12–14] HFE gene mutations may play a role in the development of significant iron overload in patients with CHC and could represent a clinically relevant comorbid factor in patients with chronic hepatitis C.[15–17]

There are several host characteristics known to affect outcome of interferon treatment, including age, gender, immune surveillance system, nutritional state and iron status.[18]

The aim of our study was to evaluate the impact of HFE gene mutations on disease severity and response to interferon therapy in a cohort of Sardinian patients with Chronic Hepatitis C.

MATERIALS AND METHODS

Patients

Sixty-nine patients with chronic hepatitis C (53 male/16 female, mean age 51 ± 2 years) were enrolled at the Division of Internal Medicine and Digestive Pathologies, University Hospital of Cagliari.

The following specific inclusion criteria were fulfilled by all patients: age 18–65 years, elevated serum ALT levels above twice the normal range for at least 6 months before enrollment; positive test for anti-HCV antibodies; positive test for HCV RNA; histological diagnosis of chronic hepatitis with or without cirrhosis.

The exclusion criteria included: decompensated liver disease, systemic diseases, cancer, hemolytic anemia, neutropenia <1000/mcl, thrombocytopenia <100 × 10^3/mcl, serological HBsAg positivity, HIV infection, drug addiction, alcohol abuse, hepatotoxic drugs usage, autoimmune hepatitis, pregnancy, psychiatric illness, renal impairment, Wilson's Disease, Hereditary Hemochromatosis and alpha-1-antitrypsin deficiency. None of the patients had received previous interferon-alpha therapy.

Liver Function Tests

Liver function tests including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (AP), g-glutamyl transpeptidase (GGT), pseudocholinesterase, bilirubin and albumin were detected.

HCV Determination

Diagnosis of hepatitis C virus infection was based on a positive anti-HCV assay (ELISA III) and quantification of Hepatitis virus C infection determination (HCV) RNA by PCR (Cobas Amplicor; Roche, Basel, Switzerland), HCV genotypes were determined by INNO-LiPA HCV II assay (Bayer Diagnostics, Leverkusen, Germany) and classified according to Simmonds et al.[19]

Iron Parameters

Quantitative determination of iron concentrations in serum was performed on automated clinical chemistry analyzers (Hitachi), using a colorimetric assay (Roche). Both ferritin and transferrin levels were measured by immunoturbidimetric assays using the Tina-quant reagents (Roche). Transferrin saturation index (TSI) was calculated as Fe/total Fe-binding capacity × 100 (normal value 16–45%).

Histological Evaluation

Liver biopsies were obtained employing the Menghini technique under ultrasound guidance in 69 patients. For histological examination, paraffin-embedded 4 mm sections were stained with hematoxylin and eosin, trichrome, and Perl's Prussian blue. Liver histology was evaluated in a blinded manner according to the Desmet classification.[20]

Genetic Analysis

Genomic DNA was isolated from either EDTA anticoagulated whole blood. Detection of C282Y, S65C and H63D mutations in the Human hemochromatosis protein (HFE) gene were performed using PCR amplification.

Study Design and Protocol

All patients were treated for 6 months (viral genotype 2/3) or 12 months (viral genotype 1/4) with pegylated-interferon (PEG-IFN) (Pegasys—Roche) 180 mcg once weekly, self-administered subcutaneously together with ribavirin (Rebetol—Schering-Plough) 1000–1200 mg/daily by body weight, orally in two divided doses.

All patients were observed every 2 weeks for the first month and every 4 weeks thereafter during treatment. After the 24–48-week therapy period, patients were followed up at 4 week intervals for 6 months. For assessment of therapy compliance, adverse effects, response to the treatment and its relationship with HFE gene mutations, patients underwent laboratory measurements of liver function tests, full blood count, serum HCV RNA concentration, thyroid