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Myeloperoxidase gene polymorphism predicts fibrosis severity in women with hepatitis C

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ABSTRACT

Oxidative stress plays an important role on liver fibrosis progression in the course of hepatitis C virus (HCV) infection. Myeloperoxidase (MPO) is an enzyme released by neutrophils and macrophages, responsible for generating hypochlorous acid and reactive oxygen species (ROS) that may lead to liver injury in HCV infection. On the other hand, antioxidant enzymes such as manganese superoxide dismutase (SOD) controls ROS-mediated damage. The aim of the present study was to investigate the influence of *MPO* G-463A and *SOD2* Ala16Val polymorphisms in the severity of liver fibrosis in individuals with chronic HCV infection. The present study included 270 patients with chronic HCV recruited from the Gastrohepatology Service of the Oswaldo Cruz University Hospital/Liver Institute of Pernambuco (Recife, Northeastern Brazil). All patients underwent liver biopsy, which was classified according METAVIR score. The SNPs were determined by real-time PCR. After multivariate analysis adjustment, the GG genotype of *MPO* and the presence of metabolic syndrome were independently associated with fibrosis severity in women ($P = 0.025$ OR 2.25 CI 1.10–4.59 and $P = 0.032$ OR 2.32 CI 1.07–5.01, respectively). The presence of the GG genotype seems to be a risk factor for fibrosis severity in women with HCV.

1. Introduction

Since its discovery in 1989, hepatitis C virus (HCV) has been reported as a major cause of chronic liver disease, cirrhosis and hepatocellular carcinoma worldwide. It is estimated that 3% of the world's population is infected with HCV, representing 170 million people. Approximately 70% of infected individuals will develop chronic liver disease, 25% will develop cirrhosis and a significant proportion will develop hepatocellular carcinoma [1,2].

The progression to severe liver fibrosis is influenced by both genetic and environmental factors. According to previous studies the major factors associated with the progression of fibrosis are older age, male sex, insulin resistance, excess of alcohol consumption and immunosuppression [3–5]. However, the pathogenesis of fibrosis remains poorly understood, due the complexity of biological factors' function involved in the fibrogenesis process [4,5].

The immune response against viral pathogens leads to the production of reactive oxygen species (ROS) by neutrophils and macrophages as a protective mechanism. Nevertheless, the overproduction of these reactive molecules combined to the decrease of cellular antioxidant defenses is responsible for creating a potentially toxic environment to the neighboring cells leading to tissue injury [6,7].

Evidences suggest that oxidative stress plays an important role on fibrosis progression with different etiology by stimulating the

Abbreviations: ALD, alcoholic liver disease; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; ER, estrogen receptor; GGT, gamma glutamyl transpeptidase; HCV, hepatitis C; HDL, high-density lipoprotein; HIV, human immunodeficiency virus; HOCl, hypochlorous acid; LDL, low-density lipoprotein; MnSOD, manganese superoxide dismutase; MPO, myeloperoxidase; MS, metabolic syndrome; NASH, nonalcoholic steatohepatitis; NF-κB, nuclear factor-κB; PPARγ, peroxisome proliferator-activated receptor gamma; ROS, reactive oxygen species; SNP, single nucleotide polymorphism.

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activation of hepatic stellate cells, myofibroblasts and genes related to the fibrogenesis [8–12].

Neutrophils and macrophages (Kupffer cells) contain myeloperoxidase (MPO), an enzyme stored in large amounts in azurophilic granules of these cells, which catalyzes the reaction between chloride (Cl^-) and hydrogen peroxide (H_2O_2) to generate hypochlorous acid (HOCl) and other ROS [13]. Together with cytokines, the local release of oxidants could cause cell death and development of hepatic fibrosis by activation of stellate cells [14–17].

To avoid tissue injury, ROS mediated-damage is regulated by a variety of anti-oxidant enzymes systems, among them one of the most important is a mitochondrial anti-oxidant enzyme, the manganese superoxide dismutase (MnSOD). MnSOD promotes the dismutation of the superoxide anion and generates hydrogen peroxide, which partly diffuses out of the mitochondria [18].

Cellular levels of MPO are influenced by a single nucleotide polymorphism (SNP) in the promoter region at position G-463A preceding the *MPO* gene. The G/A base exchange creates an SP1 transcription factor binding site in the G allele, and an estrogen receptor binding site in the A allele. The GG genotype is associated with higher expression of MPO than the GA and AA genotypes in some conditions [19,20].

Regarding the MnSOD, the polymorphism in the *SOD2* gene (Ala16Val) is associated with alterations in the MnSOD transport ability into the mitochondria. The presence of alanine (G allele) is related with more efficient mitochondrial import than valine (A allele) and higher enzyme activity [18].

Thus, the present study aimed to investigate the influence of the *MPO* G-463A and *SOD2* Ala16Val polymorphisms with the severity of liver fibrosis in patients with chronic HCV infection.

2. Materials and methods

2.1. Patients

A total of 270 patients from the Gastrohepatology Service of the Oswaldo Cruz University Hospital/Liver Institute of Pernambuco (Recife, Northeastern Brazil) were consecutively selected from August 2010 to August 2011. Patients were enrolled if they had persistent anti-HCV antibodies, were HCV-RNA positive, and underwent liver biopsy before the antiviral treatment. Presence of hepatitis A, hepatitis B, and human immunodeficiency virus (HIV) antibodies were considered as exclusion criteria. Written informed consent was obtained from all patients and a profile with clinical, biochemical and HCV genotype information was made through a questionnaire. The following clinical data were collected: age, sex, body mass index (BMI), diabetes, alcohol intake, and source of infection. Alcohol intake was considered as the alcohol consumption (>40 g/day for men and >20 g/day for women) before the patient discovered the liver disease. Metabolic syndrome (MS) was classified according the National Cholesterol Education Program's Adult Treatment Panel III (NCEP-ATP III) [21], by the presence of three or more of the following components: abdominal obesity (waist circumference >102 cm for men and >88 cm for women), triglycerides ($\geqslant$150 mg/dL), HDL cholesterol (<40 mg/dL for men and <50 mg/dL for women), blood pressure ($\geqslant$130/85 mmHg) and fasting glucose ($\geqslant$110 mg/dL).

The present study was approved by the Ethical Committee in Research of the University of Pernambuco under the protocol 47/2010 – CAAE: 0041.0.106.000-10 and was conducted in accordance with the provisions of the declaration of Helsinki and Good Clinical Practice guidelines.

2.2. Histopathology of the liver

Liver biopsies were evaluated by a single expert pathologist and assessed according to the METAVIR scoring system, which fibrosis is scored as F1 (portal fibrosis), F2 (portal fibrosis with few septa), F3 (septal fibrosis) and F4 (cirrhosis) [22]. Patients with METAVIR score F1 or F2 were classified as mild fibrosis group, those with F3 or F4 were classified as severe fibrosis group.

2.3. DNA extraction and genotyping

Genomic DNA was extracted from whole blood by using Wizard Genomic DNA Purification Kit (Promega, Madison, WI) following the manufacturer's instruction. The extracted DNA was stored at −20 °C until analyzed. Genotyping for the *SOD2* Ala16Val (rs4880) and *MPO* G-463A (rs2333227) polymorphism were performed by real-time PCR with allelic-specific TaqMan probes (Applied Biosystems, Foster City, CA). The following probes and primers were used: FAM-aggctgaggcAggtggat-TAMRA, VIC-tgaggc Gggtggatcact-TAMRA, Foward-TCTTGGGCTGGTAGTGC, Reverse-G TATTTTTAGTAGATACAGGGTTTCA. Protocol conditions are available on the SNP500 Cancer website [23]. *SOD2* Ala16Val genotyping was performed by TaqMan Genotyping Assay (Applied Biosystems, Foster City, CA).

2.4. Statistical analysis

The data was analyzed using SPSS statistical software package version 17.0 (SPSS, Inc., Chicago, IL). Categorical variables were compared using the χ^2 test or Fisher's exact test when appropriate. Kolmogorov–Smirnov test was used to check for normal distribution of continuous variables. Two-group comparisons were performed using the Student's *t* test or Mann–Whitney *U*-test for parametrically or nonparametrically distributed data. Binary logistic regression was performed to identify predictors of fibrosis severity. The results are presented using odds ratio (OR) with 95% confidence interval (CI). The differences were considered statistically significant when the *P*-value was <0.05.

3. Results

3.1. Characteristics of the patients

Clinical, biochemical and viral characteristics of the study patients are reported in Table 1. Patients with severe fibrosis were older compared to patients with mild fibrosis ($P = 0.0002$). Metabolic syndrome was significantly associated with fibrosis severity ($P = 0.003$). Serum levels of biochemical variables such as total bilirrubin, AST, GGT and alkaline phosphatase, were also, higher in patients with severe fibrosis ($P < 0.01$). However, patients with mild fibrosis presented increased levels of total cholesterol and HDL-cholesterol ($P < 0.05$). HCV genotype did not influence fibrosis stage. The frequency distribution of genotypes 1, 2 and 3 in the mild fibrosis group were 65.8%, 3.7% and 30.5%, respectively; and 71.4%, 2.6% and 26.0% in the severe fibrosis group ($P > 0.05$) (Table 1).

3.2. SOD2 Ala16Val and MPO G-463A polymorphisms and fibrosis severity

All groups assessed in this study were found to be in Hardy–Weinberg equilibrium. Tables 2 and 3 summarize genotypic and allelic frequencies of *SOD2* Ala16Val and *MPO* G-463A polymorphisms in the overall patients, men and women with chronic