Transactions of the Royal Society of Tropical Medicine and Hygiene 105 (2011) 396–400

Contents lists available at ScienceDirect

Transactions of the Royal Society of Tropical Medicine and Hygiene

journal homepage: http://www.elsevier.com/locate/trstmh

Possible role of angiotensin-converting enzyme polymorphism on progression of hepatic fibrosis in chronic hepatitis C virus infection

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ARTICLE INFO

Article history:
Received 23 June 2010
Received in revised form 18 March 2011
Accepted 18 March 2011
Available online 5 May 2011

Keywords:
Rennin-angiotensin system
HCV
Liver fibrosis
Polymorphisms
Angiotensin converting enzyme

ABSTRACT

Many functional polymorphisms in the rennin-angiotensin system (RAS) have been described; these polymorphisms have been postulated to contribute to fibrosis in several diseases. Our aim was to study the frequency of ACE I/D polymorphism in chronic hepatitis C virus (HCV) infection and its association with liver fibrosis and response to treatment. This study included 90 patients with chronic hepatitis C. All patients received antiviral therapy in the form of pegylated interferon and ribavirin. Patients were grouped according to the stage of liver fibrosis by biopsy into: group 1 (fibrosis: 0 or 1); group 2 (fibrosis: 2 or 3) and group 3 (fibrosis: 4 or 5). The study included also 170 healthy subjects, as a control group. Polymerase chain reaction was carried out to detect the different ACE genotypes. The D/D genotype was significantly more prevalent among HCV patients compared to controls (65.6% vs 48.2%, $P = 0.006$). Degree of necroinflammation was significantly higher among patients with I/I genotype when compared to patients with D/D genotype ($P = 0.04$). No significant difference in the distribution of the ACE I/D genotypes between the fibrosis groups and between responders and non responders to interferon therapy. The D/D genotype may increase the susceptibility to infection with hepatitis C.

1. Introduction

The prevalence of hepatitis C virus (HCV) infection varies throughout the world, with the highest number of infections reported in Egypt. The use of parenteral antischistosomal therapy in Egypt is thought to have contributed to a prevalence of antibodies against HCV in various regions ranging from 6–28% (mean 22%). In the United States, 1.8% of the population is positive for HCV antibodies. In Europe, the general prevalence of HCV is about 1% but varies among the different countries.[1]

Progressive hepatic fibrosis and cirrhosis develops in 20–30% of patients with chronic HCV infection.[2] Several host and environmental factors affect the rate of progression to fibrosis, such as male gender, excessive alcohol consumption, age of acquisition of infection and obesity.[3,4] In addition, the variability in disease progression is influenced by host genetic factors. Many functional polymorphisms in the rennin-angiotensin system (RAS) have been described and these polymorphisms have been postulated to contribute to organ damage and fibrosis in several diseases.[5,6] One of these polymorphisms is the

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doi:10.1016/j.trstmh.2011.03.005

angiotensin-converting enzyme (ACE) I/D gene polymorphism. The D variant of this polymorphism is characterized by absence of insertion within intron 16 in the gene and this is associated with increased ACE and angiotensin II levels.[7]

Previous studies reported that RAS plays an important role in liver fibrosis. Angiotensin Π increases DNA synthesis and proliferation of hepatic stellate cells (HSCs) in a dose-dependent manner, also it promotes contraction and proliferation of myofibroblast. Furthermore angiotensin Π promotes the release of inflammatory cytokines as well as the deposition of extracellular matrix.[8,9] It has been reported that clinically used ACE inhibitor and angiotensin I receptor blocker significantly attenuated liver fibrosis development in experimental models.[10] In humans, it has been reported that RAS inhibitory agents have antifibrotic effects in several types of chronic liver diseases including HCV.[11,12]

The aim of this study is to study the frequency of ACE I/D polymorphism in chronic HCV infection and its association with liver fibrosis and response to treatment among Egyptian patients with chronic hepatitis C.

2. Patients and methods

2.1. Patients

The study was conducted on 90 patients with documented chronic HCV infection, recruited from National Liver And Tropical Diseases Institute, Cairo and from Tanta University Hospital. Chronic HCV was diagnosed based on elevated serum transaminase levels for at least six months and positive HCV antibody by the second-generation enzyme-linked immunosorbent assay and confirmed by detection of circulating HCV RNA using polymerase chain reaction (PCR). A liver biopsy specimen was taken from every patient. The study also included 170 healthy subjects, age and sex matched with the patients, as a control group. All controls were negative after anti HCV Ab screening test. Patients with other diseases like diabetes mellitus, hypertension or cardiovascular disease, and also patients with other causes of chronic liver diseases were excluded. None of the patients were receiving ACE-inhibitors or angiotensin II receptor antagonists.

All patients received interferon alpha therapy for at least six months, except for two patients who discontinued treatment early due to adverse effects.

2.2. Histological evaluation

Liver biopsy was obtained from all patients under ultrasound guidance. Biopsies were stained with hematoxylin and eosin and liver specific stains including van Gieson and Masson's Trichrome. All biopsies were evaluated by the same pathologist blinded to the clinical and laboratory criteria. Grading for the degree of necroinflammatory activity and staging for the extent of fibrosis were done according to the criteria of Ishak et al.[13] Patients were grouped according to the stage of fibrosis into group 1: fibrosis score 0, 1; group 2: fibrosis score: 2, 3; and group 3: fibrosis score 4, 5.

2.3. Molecular analysis of the angiotensin-converting enzyme (ACE) I/D polymorphism

Genomic DNA of patients and normal control subjects was extracted from peripheral blood lymphocytes using the salting out method.[14] PCR amplification of intron 16 of the ACE gene entailing the I/D polymorphism was performed as described by Patil et al., 2005[15] using the following primer sequences:

5'-CTGGAGACCACTCCCATCCTTTCT-3' and

5'-GATGTGGCCATCACATTCGTCAGAT-3'.[14] PCR was carried out in a 25 μl total volume containing 0.5 μg genomic DNA, 10 mM TrisHCL (pH = 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.6 μM of each primer and 2 units of Taq DNA polymerase (Finnzymes, Espoo, Finland). PCR conditions were: initial denaturation at 96 °C for 10 min followed by 35 cycles of denaturation at 95 °C for 1 min, annealing at 59 °C for 1 min and extension at 72 °C for 2 min. Amplified PCR products were analyzed on 2% agarose gel. Amplification with this primer pair results in 490 bp and 190 bp amplification products corresponding to the I and D alleles respectively.

2.4. Statistical methods

Statistical analysis of the data was performed using Sigma-Stat version 2.03 (Systat Software, Inc., CA, USA) and SPSS version 14 (SPSS Inc., Chicago, IL, USA). Results were expressed as the mean ± SD. Allele frequency was calculated by the allele counting method, which was then utilized to determine the allele frequency. Differences between nominal variables were analyzed by χ^2 tests. Two-tailed P-value < 0.05 was considered statistically significant.

3. Results

3.1. Patients' characteristics

Ninety patients with chronic HCV were evaluated, 68 males and 22 females; their mean age was 42.96 ± 8.74 years. The stage of fibrosis ranged from 0 to 5; the mean value of necroinflammatory score was 7.38 ± 3.88.

All patients received antiviral therapy in the form of pegylated interferon and ribavirin; 51 (57.9%) patients showed sustained virological response while 37 (42.1%) were non responders. The frequencies of fibrosis stages 0–1, 2–3 and 4–5 were 25.5%, 41.2% and 33.3% respectively. These frequencies showed no significant differences. The I allele frequency among patients was 22.2% while in controls was 29.4%. The D allele frequency among patients was 77.8% while in controls was 70.6%. There was no significant difference in allele frequency between patients and controls.

The genotypes were detected using PCR and agarose gel electrophoresis (Figure 1). The frequency of the I/I, I/D and D/D genotypes among patients was 10.0%, 24.4% and 65.6% compared to 7.1%, 44.7% and 48.2% respectively in the control group. This showed that there is a significant increase of