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Single-nucleotide polymorphism in the promoter region of the osteopontin gene at nucleotide −443 as a marker predicting the efficacy of pegylated interferon/ribavirin-therapy in Egyptians patients with chronic hepatitis C

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ABSTRACT

Osteopontin (OPN) is an extracellular matrix glycophosphoprotein produced by several types of cells including the immune system. The present study examined the possibility that single-nucleotide polymorphisms (SNP) in the promoter region of the OPN at nt −443 is a marker predicting the therapeutic efficacy of pegylated interferon (peg-IFN-α2b)–ribavirin combination therapy in Egyptian patients with chronic hepatitis C. Blood was collected from 95 patients with chronic hepatitis C who had received peg-IFN-α2b–ribavirin combination therapy and 100 age and sex matched controls. SNP in OPN at nucleotide (nt) −443 and its serum protein level were analyzed. Sustained virological response (SVR) was higher in patients with T/T at nt −443 than in those with C/C or C/T. A univariate logistic regression analysis showed that fibrosis grade, serum OPN protein level and T/T homozygotes of SNP at −443 were significant predictors for response. Receiver operating characteristics (ROC) analysis revealed the diagnostic and prognostic efficacy of serum OPN. It can be concluded that SNP in the promoter region of OPN at nt −443 and serum OPN protein level are predictors of response to the efficacy of peg-IFN-α2b–ribavirin therapy in Egyptian patients with chronic hepatitis C.

1. Introduction

Hepatitis C virus (HCV) infection is a global health problem, because it is estimated to prevail in 170 million people worldwide [1]. Egypt has the largest epidemic of HCV infection in the world. The recently released Egyptian Demographic Health Survey [EDHS] tested a representative sample of the entire country for HCV antibody and revealed that the overall prevalence (percentage of people) positive for antibody to HCV was 14.7% [2]. The persistent viremia occurring due to HCV infection in 60–80% of such people [3,4] frequently produces chronic inflammation in the liver, hepatocyte necrosis due to cytotoxic T lymphocyte infiltration due to the T-helper (Th) 1 immune reaction, [5] and extracellular matrix (ECM) deposition in the space of Disse [6] and leads to gradual progression to liver fibrosis and cirrhosis 20–30 years after infection [7]. The incidence of hepatocellular carcinoma increased with the degree of hepatic fibrosis in patients with chronic hepatitis C and the annual incidence was 7.9% in patients with liver cirrhosis [8].

Therefore, antiviral therapies, with pegylated interferon (peg-IFN) alone or in combination with ribavirin, are required to reduce the risk of carcinogenesis in patients with chronic hepatitis C [8]. Ribavirin is a synthetic guanosine analog (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide) with a broad spectrum of anti-viral activity. It increases the ratio of patients who clear HCV RNA during therapy, and markedly reduces relapse rates in these patients when added to IFN-α [9]. Ribavirin is a prodrug, which when metabolized resembles purine RNA nucleotides that interfere with RNA metabolism required for viral replication [10]. It is known that the efficacy of IFN monotherapy or IFN–ribavirin combination therapy (IFN-based therapies) depends on the HCV genotype and serum HCV-RNA level; genotype 4a, the predominant genotype of

Abbreviations: ALB, albumin; AFP, alpha fetoprotein; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALPS, autoimmune/lymphoproliferative syndrome; CI, confidence interval; DB, direct bilirubin; ECM, extracellular matrix; ELISA, enzyme-linked immunosorbent assay; HCV, hepatitis C virus; HBc, hepatitis B core antigen; HBsAg, hepatitis B surface antigen; HIV, human immunodeficiency virus; HSC, hepatic satellite cell; IFN, interferon; IL, interleukin; OPN, osteopontin; peg, pegylated; PCR, polymerase chain reaction; PPV, positive predictive value; NPV, negative predictive value; PT, prothrombin time; ROC, receiver operating characteristics; SVR, sustained virological response; TB, total bilirubin; Th1, T-helper.

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HCV in Egypt, had poorer responsiveness to IFN-based therapies than other genotypes. It is also known that the efficacy of IFN-based therapies may be influenced not only by viral factors but also by host factors, such as the Th1 immune reaction [11].

Osteopontin (OPN) is an ECM glycophosphoprotein with an arginine–glycine–aspartate (RGD) motif, that is produced by cells of the immune system such as macrophages, epithelial cells, smooth muscle cells, osteoblasts and cancer cells [12,13]. OPN protein is produced in virtually all tissues and appears in a variety of biologic fluids including blood, urine, milk, and seminal fluid [12]. It is involved in a multitude of physiologic and pathologic events, e.g., angiogenesis, apoptosis, inflammation, wound healing and tumor metastasis [14].

Overexpression of OPN has been described in several conditions in which basic inflammatory processes are activated, such as myocardial remodeling after infarction [15] and kidney interstitial fibrosis after obstructive uropathy [16]. Increased serum OPN levels have also been associated with several autoimmune diseases, including multiple sclerosis [17], rheumatoid arthritis [18], and autoimmune/lymphoproliferative syndrome (ALPS) [19], as well as a variety of cancers [20].

Previously, OPN was found to be expressed in activated Kupffer cells and hepatic stellate cells (HSCs), and to contribute to the migration of macrophages into necrotic areas in injured rat liver [21,22]. Genetic polymorphisms in the OPN gene were reported to determine the magnitude of the immune reaction to bacterial infection in mice [23] and to be associated with several diseases, including autoimmune diseases such as lupus erythematosus [24], multiple sclerosis [25], and ALPS [19].

Four single-nucleotide polymorphisms (SNPs) in the promoter region of human OPN (nt −155, −443, −616, and −1748) were found, and it has been suggested that SNP in OPN at nucleotide (nt) −443 (C or T) was a novel one that affected hepatitis activity in patients with chronic hepatitis C [22,26]. From these observations, we assumed that SNP in the promoter region of OPN at nt −443 might be a marker to predict the efficacy of IFN-based therapy in chronic HCV-infected patients.

In the present study, we analyzed SNP in the promoter region of OPN at nt −443 in Egyptian patients with chronic hepatitis C treated with peg-IFN-α2b combined with ribavirin, and evaluated the significance of this SNP and its serum level as a marker predicting the efficacy of this therapy.

2. Materials and methods

2.1. Patients

This prospective study was carried out on 95 Egyptian patients chronically infected with HCV (male = 53 and female = 42), mean age 38.8 ± 6.73 years; range: 20–52 years, who were admitted at the outpatient's Clinic of the liver unit, Tropical Medicine Department, at Kasr El-Aini Hospital, Cairo University, Cairo, Egypt, from March 2009 to April 2010 to receive IFN-based therapy combined with ribavirin.

All the patients were positive for HCV-RNA in the sera for more than 6 months before the therapy. All patients were newly diagnosed and none had received any form of interferon based therapy or hepatoprotective treatment before collection of blood samples for biochemical analysis. The diagnosis of chronic hepatitis C was made by histological findings in liver biopsy specimens and by serum biochemical tests and peripheral blood cell counts.

Subjects were excluded from the study if they had active schistosomiasis, hypertension, hepatitis B virus (HBV) or human immunodeficiency virus (HIV) infection, any disease other than chronic HCV infection, history of alcohol abuse, renal insufficiency, proteinuria, suspected infections, clinically overt diabetes mellitus, thyroid dysfunction, or any other endocrine disorder and autoimmune liver disease by presence of ANA titer (antinuclear antibodies) > 1/160.

Table 1
Demographic and clinical features in controls and chronic hepatitis C patients.

Variables	Controls	Patients	*P*-value
n	100	95	
Sex			
Females % within group	55 (55%)	42(44.21%)	0.38
Males % within group	45 (45%)	53(55.79%)	
Age (years)	36.40 ± 7.29	38.80 ± 6.73	0.16
ALT (U/L)	30 ± 6.01	86.93 ± 46.02	<0.001*
AST (U/L)	30.2 ± 6.10	106.08 ± 69.03	<0.001*
ALP (U/L)	43.65 ± 7.57	118.16 ± 28.64	<0.001*
TB (mg/dL)	0.74 ± 0.20	1.19 ± 0.60	<0.001*
DB (mg/dL)	0.15 ± 0.06	0.34 ± 0.27	<0.01*
ALB (g/dL)	11.19 ± 0.62	3.53 ± 1.67	<0.01*
PT(seconds)	11.19 ± 0.62	12.50 ± 1.67	<0.01*
AFP (ng/ml)	5.86 ± 2.03	13.92 ± 9.51	<0.001*
HCV-RNA (IU/ml)	0	$40 \times 10^6 \pm 15 \times 10^7$	<0.03*

Data are represented as means ± standard deviation (SD).
* Indicate statistical significance.

A total of 100 healthy control volunteers were matched to the patient population by sex (male = 45, female = 55) and mean age (36.4 ± 7.29 year, range: 21–49 years). None of them had a history of hepatic diseases or endocrine disorders; all had completely normal liver function tests, normal ultrasound of the liver and biliary system, and negative serological findings for viral and autoimmune liver diseases and diabetes. Clinical data of the patients and normal controls are given in Table 1. An informed consent for gene analysis was obtained from all enrolled subjects in this study. The study protocol was approved by the ethics committee of the Faculty of Pharmacy, Cairo University and conformed to the ethical guidelines of the 1975 Helsinki Declaration.

2.2. Treatment regimens

Each patient received subcutaneous injection of peg-IFN-α2b, at 1.5 μg/kg body weight once a week, combined with daily oral administration of ribavirin at 600, 800, or 1000 mg/day for patients with a body weight of less than 60 kg, between 60 and 80 kg, and more than 80 kg, respectively, for 48weeks. The intervals between peg-IFN-α2b injections, and the doses of peg-IFN-α2b and ribavirin were changed when severe side effects occurred.

2.3. Blood sampling and laboratory assays

Venous blood samples (~10 ml) were collected by trained laboratory technicians and complete blood picture (CBC) was performed. A portion of blood was allowed to clot and then centrifuged at 3500 rpm for 5 min to separate the serum used for assessment of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) activities, total bilirubin(TB), direct bilirubin(DB), HCV-RNA, HCV specific antibody titers, alpha-fetoprotein (AFP) and OPN levels. Another portion of blood was collected in vacutainer tubes containing citrate to separate plasma used for assay of albumin (ALB) and prothrombin time (PT). Assays were performed using Roche Hitachi Chemistry Analyzer (USA). A second portion of blood collected in EDTA containing vacutainer tubes was stored at −80 °C until molecular assays.

Liver function tests and CBC were done at weeks 1, 2, 4 and repeated monthly thereafter during treatment to detect the development of any adverse side effects to the drugs necessitating dose modification and/or temporary or permanent stoppage of treat-