Prevalence of the -308 and -238 Tumor Necrosis Factor Alpha (TNF-α) Promoter Polymorphisms in Mexican Chronic Hepatitis C Patients

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Abstract

Background: *Tumor necrosis factor alpha (TNF-α) has been involved in the pathogenesis of chronic hepatitis C virus (HCV) infection. Two polymorphisms at positions -308 and -238 in the TNF-α promoter region influence TNF-α expression and these have been linked to a number of infectious diseases.*

Aim: *To analyze the prevalence of the -308 and -238 TNF-α polymorphisms in a group of Mexican HCV-infected patients and in healthy control subjects not related to the patients.*

Material and methods: *Both polymorphisms were determined in peripheral blood samples from 48 patients with positive anti-HCV antibodies and discernible HCV-RNA levels. Twenty-five patients were women and 23 were men. The control group included 100 healthy subjects. Forty-four were women and 56 were men. The polymorphisms were evaluated by polymerase chain reaction amplification (PCR), followed by the Restriction Fragment Length Polymorphism (RFLP) method.*

Results: *The prevalence of the -308 TNF-α polymorphism was found to be 12% in patients with chronic hepatitis C and 20% in control subjects, (P = 0.2616); whereas that of the -238*

Resumen

Antecedentes: El factor de necrosis tumoral alfa (TNF-α) se ha involucrado en la patogénesis de la hepatitis crónica producida por el virus de la hepatitis C (VHC). Dos polimorfismos en la posición -308 y -238 de la región promotora del gen TNF-α influyen en la expresión de TNF-α y se han asociados con numerosas enfermedades infecciosas.

Objetivo: Analizar la prevalencia de los polimorfismos -308 y -238 de TNF-a en una población mexicana de pacientes infectados con VHC y controles sanos no relacionados.

Material y métodos: Se determinaron ambos polimorfismos en muestras de 48 pacientes con anticuerpos anti-VHC positivos y niveles detectables de VHC-RNA (25 mujeres y 23 hombres) y 100 sujetos sanos (44 mujeres y 56 hombres). Los polimorfismos se evaluaron por reacción en cadena de la polimerasa (PCR), seguido del método de polimorfismos en la longitud de los fragmentos de restricción (RFLP).

Resultados: La prevalencia del polimorfismo -308 de TNF-a fue 12% en los pacientes con hepatitis C crónica y 20% en los sujetos control (P = 0.2616); mientras que la prevalencia del polimorfismo -238 TNF-a fue 2% en los pacientes con VHC y 12% en

TNF-α polymorphism was found to be 2% and 12% in patients and control subjects, respectively (Π = 0.061). The TNF-α genotypes were found to be in Hardy-Weinberg equilibrium.
Conclusions: *No association was found between -308 and -238 TNF-α polymorphisms and chronic hepatitis C in the Mexican group studied. Our data suggest that additional studies increasing the sample size and a control group which has been exposed to an equal risk of infection are required to investigate whether these polymorphisms represent genetic susceptibility for chronic HCV infection.*

Key words: *viral hepatitis, chronic hepatitis C, tumor necrosis factor alpha, genetic polymorphism, polymerase chain reaction*

los controles (P = 0.061). Los genotipos estuvieron en equilibrio de Hardy-Weinberg.
Conclusión: No se encontró ninguna asociación entre los polimorfismos -308 y -238 de TNF-α con hepatitis C crónica en la población mexicana estudiada. Se necesitan estudios adicionales para investigar si estos polimorfismos representan una susceptibilidad genética para la infección por VHC.

Palabras clave: hepatitis viral, hepatitis crónica C, factor de necrosis tumoral alfa, polimorfismos genéticos, reacción en cadena de polimerasa.

Introduction

Infection by Hepatitis C Virus (HCV) causes chronic liver disease and is a worldwide health problem, affecting 3% of the world's population.[1,2] It has been reported that cytokines play key roles in the response to viral infections and a number of them have been associated with the pathogenesis of HCV infection.[3] Tumor necrosis factor alpha (TNF-α) is a pro-inflammatory cytokine that has been associated with HCV infection.[4] Several studies have shown that serum and messenger ribonucleic acid (mRNA), levels of TNF-α and soluble TNF receptors (sTNFR) may correlate with histological severity and/or lack of response to therapy in chronically ill HCV-infected patients.[5-7] However, these data have been inconsistent because Falasca *et al*.[8] reported that TNF-α and interleukin-2 (IL-2) serum levels were higher in hepatitis B virus patients in comparison to HCV positive patients ($P < 0.001$). Additionally, the level of sTNFR has been proposed as a good indicator of TNF production; therefore the sTNFR level is more valuable for monitoring the degree of TNF-α system activity than the TNF-α level.[9]

G to A polymorphic sequence at position -308 (TNF1 to TNF2 allele) and -238 (TNFG to TNFA allele) in the TNF-α promoter has been shown to influence TNF-α gene expression.[10,11] Moreover, these polymorphisms have been associated with clinical characteristics of chronic hepatitis C.[12,13] However, conflicting data with regard to the association between TNF-α promoter polymorphisms and the progression of chronic hepatitis C or response to interferon (INF) therapy have been reported.[14,15] Therefore, the aim of this study was to analyze the prevalence of the -308 and -238 TNF- polymorphisms in a group of Mexican HCV-infected patients and control subjects not related to the patients.

Material and methods

Patients and Control Subjects

We studied 48 HCV-infected patients (25 women and 23 men, mean age 45 ± 12 years) being treated at the Infectious Diseases Service and Liver Unit at the University Hospital in Monterrey and community-based out-reach facilities. HIV/HCV co-infected patients and pregnant woman were not included in this study. The aforementioned public hospital is the largest in northeastern Mexico with patients coming principally from the state of Nuevo León and surrounding states (Coahuila, Tamaulipas and San Luis Potosí). HCV infection was diagnosed on the basis of clinical, biochemical and serological data. Demographic and histological data and liver function test results were collected from all of the patients. The analysis of anti-HCV antibodies, HCV-RNA in plasma and

HCV genotyping was performed according to the methods previously described.[16] The research protocol was reviewed and approved by the hospital review boards. All participants signed informed consent forms at enrollment.

The control group included 100 healthy subjects who tested negative for HIV and HCV and other infectious diseases at the time of enrollment contemporaneously with case subjects. A peripheral blood sample was collected from each one. Forty-four were women and 56 were men. Mean age was 28 ± 11 years. The research protocol was reviewed and approved by institutional review boards. At enrollment, all participants signed the informed consent forms.

DNA isolation

Genomic deoxyribonucleic acid (DNA) was obtained from Ethylene-Diamine-Tetraacetic- Acid (EDTA)-preserved whole blood samples from patients and control subjects using the phenol-chloroform method as described before.[17]

Identification of TNF-α promoterpolymorphisms

Polymorphisms were detected by polymerase chain reaction (PCR) followed by the Restriction Fragment Length Polymorphism (RFLP) method. For polymorphism of TNF-a promoter at position -238 we used the following primers; sense primer 5'-AGACCCCCCTCGGAACC-3' and antisense primer 5'-ATCTGGAGGAAGCGGTAGTG-3'.[18] while for -308 TNF-alpha polymorphism we used the sense primer 5'-CAATAGGTTTTGAGGGCCAT-3' (modified from Day et al. 1998).[18] and the antisense primer described above. These primers containing a single base-pair mismatch adjacent to the polymorphism site to introduce a restriction site for the *Msp I* **and** *Nco I* enzymes (New England BioLabs, Beverly, MA, USA) into the wild-type nucleotide sequences after amplification for detecting the -238 and -308 polymorphisms, respectively.11,19 The amplified products of 151 bp and 223 bp were digested with *Msp I* and *Nco I* restriction enzymes and analyzed using 2% agarose gel.

Statistical Analysis

Descriptive statistics was used (mean and standard deviation). Hardy-Weinberg equilibrium was evaluated for these two polymorphisms within each group by the X^2 test. Statistical significance was defined as $P < 0.05$. Data analysis was performed using the Epi-Info 2000 software version 3.3.2 and the SPSS version 13.0.

Results

We analyzed the frequency of -308 and -238 TNF-α polymorphisms in 48 patients with chronic HCV infection and 100 healthy non-related control subjects. The baseline characteristics of the patients with chronic HCV infection and healthy control subjects are given in **Table 1**. We also identified the HCV genotypes in these patients where the predominant genotype was 1 (79%), followed by genotype 2 (13%), 3 (6%) and 4 (2%) **(Table 1)**. These results are similar to those reported previously for patients from northeastern Mexico and other regions of Mexico.[16,20]

The genotype frequency of the -308 and -238 TNF-α polymorphism (with the prevalence of TNF2 and TNFA alleles) in patients with chronic hepatitis C infection and healthy control subjects is shown in **Tables 2 and 3**. The genotype frequencies of all of these polymorphisms did not deviate significantly from Hardy-Weinberg equilibrium in the two groups.

The prevalence of the -308 TNF-α polymorphism was 12% in patients with chronic hepatitis C and 20% in control subjects, respectively ($P = 0.2616$) **(Table 2)**.

Discussion

Dai et al.[13] reported that the TNF-α promoter genotype at the position -308 appears to be associated with variability in severity of fibrosis and viral load in chronic HCV infection; nevertheless, we did not find a significant difference in the frequency of this polymorphism between these two groups. On the other hand, the prevalence of the -238 TNF-α polymorphism was 2% in HCV patients and 12% in control subjects ($P = 0.061$). Hohler et al.[12] reported that TNF-α gene promoter -238 polymorphisms were associated with chronic active hepatitis caused by HCV; however, no significant difference in the frequency of this polymorphism was observed between patients and the control group.

In addition, a previous study of the distribution of allele variants of the TNF-α gene carried out by German researchers failed to detect significant differences in the content of G-308A polymorphism genotypes in healthy subjects and patients infected with HCV (but they found allele associations

Table 1.
Demographic and clinical characteristics of HCV patients and control subjects

Characteristics *n* (%)	HCV patients *n* = 48 *n* (%)	Control subjects *n* = 100
Age (mean ± SD)	45 ± 12	28 ± 11
Gender		
Female	25 (52)	44 (44)
Male	23 (48)	56 (56)
Hepatic enzymes		
AST		
0-1	4 (8)	100 (100)
***1-3**	36 (75)	-----
***> 3**	8 (17)	-----
ALT		
0-1	7 (14)	100 (100)
***1-3**	31 (65)	-----
***> 3**	10 (21)	-----
HCV genotyping		
1	38 (79)	-----
2	6 (13)	-----
3	3 (6)	-----
4	1 (2)	-----

* Patients with abnormal values: number of times above upper limit normal.
AST: aspartate aminotransferase, ALT: alanine aminotransferase

Table 2.
Frequency of the -308 TNF-**a** polymorphism (TNF2 allele).

Genotype Value	*n*	Genotype	Allele	*n*	Allele
HCV positive	42	88	TNF1	90	94
TNF1/TNF 1	0.2616 6	12	TNF2	6	6
TNF1/TNF 2	0 48	100		96	100
TNF2/TNF 2	20 0	80 20	TNF1 TNF2	180 20	90 10
Total CONTROL	0 100	0 100		200	100
TNF1/TNF					

Table 3.
Frequency of the -238 TNF-α polymorphism (TNFA allele).

Genotype P-Value	*n*	Genotype frequency (%)	Allele	*n*	Allele frequency (%)
HCV positive	47	98	TNFG	95	99
TNFG/TNFG	0.060				
TNFG/TNFA	1	2	TNFA	1	1
TNFA/TNFA	0	0			
Total Control	48	100		96	100
TNFG/TNFG	88	88	TNFG	188	94
TNFG/TNFA	12	12	TNFA	12	6
TNFA/TNFA	0	0			
Total	100	100		200	100

with the disease for transition in locus -238).[12] No appreciable differences in the incidence of TNF-α allele -308 were detected in a group of HCV infected patients belonging to different races.[12,13] Previous studies found no association between TNF-α gene polymorphism in HCV patients (belonging to different races) and response to combined or monotherapy with IFN.[12,13]

Current reports indicate that TNF- effects on the course and outcome of HCV infection deserve further in vivo and in vitro investigations. The presence of a certain set of allele variants of

cytokine genes, for example TNF- gene located in promoter regions, and hence, determining the level of spontaneous and inducible IL production by cells can be essential for the outcome of host contact with HCV and for the course of the infectious process and the efficiency of cytokine therapy.

In our study, the analysis of TNF-α promoter polymorphisms indicates that there was no significant difference between HCV infected patients and control subjects. These results are contrary to other reports showing that TNF-α promoter polymorphisms are associated with chronic hepatitis C.[12,13]

In conclusion, our results indicate absence of relationship among these TNF-α polymorphisms and occurrence of hepatitis C, suggesting that other factors are involved in the progression of this disease. However, functional studies in a larger population characterizing TNF-α transcriptional activity in CHC patients carrying -308 and -238 TNF-a variant allele(s) may help to clarify the role of these promoter polymorphisms in susceptibility to HCV infection.

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Acknowledgments

We are especially grateful to the medical and technical staff at the Liver Unit of the Internal Medicine Department at the University Hospital of UANL. This study was supported by a grant from CONACYT (SALUD-2003-C01-002) awarded to AM Rivas-Estilla.