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Short Communication

HPA-1a/1b could be considered a molecular predictor of poor prognosis in chronic hepatitis C

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Abstract

Introduction: HPA polymorphism has been associated with HCV presence and fibrosis progression in chronic hepatitis C. However, it is unknown if there is an association between HPA-1 polymorphism and hepatocellular carcinoma (HCC). Therefore, this study aimed to evaluate HPA-1 polymorphism in the presence of HCC. **Methods:** PCR-SSP was used to perform HPA genotyping on 76 HCV-infected patients. **Results:** There was no association between patients with and without HCC. There was significant difference in HPA-1 genotypic frequency distribution between HCC and F1/F2 fibrosis degree. **Conclusions:** The HPA-1a/1b polymorphism appears to be more associated with liver damage progression than with HCC presence.

Keywords: Hepatitis C virus. Human platelets antigens. Hepatocellular carcinoma.

Hepatocellular carcinoma (HCC) develops in 1% to 5% of patients with chronic hepatitis C as a consequence of disease progression. In many cases, HCC is detected approximately 30 years after the initial hepatitis C virus (HCV) infection. The presence of HCC is indicative of both progressive injury of liver function[1] and an advanced disease stage.

Although, previous studies have demonstrated that development of HCC in patients with chronic hepatitis C is influenced by age, sex, and alcohol abuse[2], host genetic polymorphisms may also have contribute to HCC development. Additionally, human leukocyte antigen (HLA)[3], single nucleotide polymorphisms (SNPs) in tumor necrosis factor-α (TNF-α)[4], and deletions in glutathione S-transferase (GST) genes[5] have been associated with the presence of HCC in patients with chronic hepatitis C. Further, previous studies have demonstrated that the levels of some integrins are altered in HCC[6].

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Integrins are transmembrane proteins expressed in several cell types, including some liver cells and platelets[7]. Previous studies have shown that changes in amino acid sequences in integrin can alter protein conformation, leading to impaired integrin function. Some integrins present in hepatic stellate cells (HSCs) and platelets express polymorphic antigens that are referred to as human platelet antigens (HPA)[8]. There are several HPA systems and the majority of these display single nucleotide polymorphisms, where a change in one nucleotide modifies the corresponding amino acid[9].

Similar to HLA polymorphism[3], HPA polymorphisms have also been associated with HCV presence[10], and the HPA-1 system has been specifically associated with fibrosis progression in chronic hepatitis C[11]. However, it is unknown if there is an association between the HPA-1 polymorphism and HCC presence in patients with chronic hepatitis C. In this context, the objective of this study was to evaluate a possible association between the HPA-1 polymorphism and the presence of HCC in chronic hepatitis C.

Aliquots of EDTA-anticoagulated peripheral venous blood were collected from 76 patients with chronic hepatitis C at the Department of Internal Medicine, Gastroenterology Division,

Botucatu School of Medicine, Sao Paulo State University, UNESP, Botucatu, SP, Brazil. Inclusion criteria were as follows: the presence of HCC due to HCV infection and signed informed consent. Exclusion criteria were HBV or HIV positive serology and the presence of other hepatic diseases. Characteristics of patients included in this study are described (**Table 1**). This study was approved by the Botucatu Medical School Research Ethics Committee, UNESP, in keeping with the Helsinki Declaration of 1964, as revised in 1975, 1983, 1989, 1996, and 2000.

Clinical data (sex, age, time of infection and alcohol use) were obtained from the patient's medical records. Time of infection was defined as the time elapsed between the presumed date of infection and the date of biopsy (information evaluated in 69 patients). Alcohol abuse was considered more than 40 g per day for females and more than 80 g per day for males (this parameter was evaluated in 54 patients).

Genomic DNA was isolated from whole blood using Axyprep™ DNA Extraction Kit (Axygen Scientific, Union City, California), according to the manufacturer's instructions. HPA-1 was genotyped using PCR sequence specific primers according Klüter et al. (1996)[12].

Plasma RNA, isolated using QIAamp Viral RNA Mini Kit (QIAGEN, Valencia, CA, USA), was used as the source for amplification of the HCV 5'UTR region via RT-PCR, as per Garson et al. (1990)[13]. Obtained sequences were genotyped using HCVBlast available at The Los Alamos HCV sequence database[14].

Hardy–Weinberg equilibrium test was carried out to determine the distribution of HPA-1 gene frequencies according to HCC presence. Fisher's exact test was used to verify possible association between HPA-1 alleles and HCC presence. Analysis was performed using 3 groups with distinct characteristics as controls: (a) HCV-infected patients without carcinoma, selected from a data bank developed by Verdichio-Moraes et al. (2009)[10]; (b) patients with chronic hepatitis C, without HCC and with lower stage fibrosis (F1 or F2); and (c) patients with chronic hepatitis C, without HCC and with advanced fibrosis (F3 or F4)[11]. Fisher's exact test was used to evaluate associations between the viral genotype, alcohol abuse, genotypic frequency of the HPA-1 system, time of infection, and HCC presence.

The odds ratio (OR) was calculated for parameters that showed significant associations and the confidence interval was obtained using a logistic regression model. The level of significance for all statistical tests was set at $P < 0.05$. Data were analyzed using R software version 3.2.0[15].

The results from the Fisher's exact test demonstrated that HCC presence was not associated with HCV genotype, alcohol abuse, or race (data not shown). Additionally, there were no differences in allele and genotype frequency distribution for the HPA-1 system between groups with and without HCC (data not shown) in HCV-infected patients with advanced fibrosis (F3/F4).

The results obtained demonstrated that there was no deviation from the Hardy-Weinberg equilibrium in HPA-1 systems when HCV-infected patients with HCC and HCV-infected patients without HCC and with moderate fibrosis (F1/F2) were compared (p=0.08). Further, there were no significant differences in allele frequency distribution for the HPA-1 system between these groups (**Table 2**).

On the other hand, there was significant difference (p=0.013) in HPA-1 genotypic frequency distribution between HCV-infected patients with HCC and HCV-infected patients with moderate fibrosis degree (F1/F2) (**Table 2**). The frequency of the HPA-1a/1b genotype was significantly higher in patients with HCC compared to that of HCV-infected patients with moderate fibrosis (F1/F2), as indicated by an odds ratio of 0.2711 (95% CI - 0.0899–0.729) (**Table 3**).

The progression of chronic hepatitis C can lead to HCC development. Although HCC development is often described as

TABLE 1: Characteristics of patients with hepatocellular carcinoma resulting from hepatitis C virus (HCV) infection.

Characteristic	(N = 76)
Age, Years [median (IQR)][a]	60.00 (54.75 - 64.0)
Sex, Male [N (%)][a]	67 (88.15)
Genotype HCV [N (%)]	
Genotype 1	38 (50.00)
Genotype 3	36 (47.38)
Genotype 1 and 3	01 (1.31)
No genotyping[b]	01 (1.31)
HCV Viral Load[a,c]	
Undetectable	13 (17.11)
Detectable	56 (73.68)
No information	07 (9.21)
Alcohol Abuse[e]	
Yes	21 (27.63)
No	33 (43.42)
No information[d]	22 (28.95)
Race [N (%)][a]	
Caucasian	57 (75.00)
Black	04 (5.27)
Others (Metis or Asian)	06 (7.89)
No information[d]	09 (11.84)

IQR: interquartile range. [a] information obtained from patients' medical records. [b] HCV genotyping could not be performed due to technical limitations. [c] Undetectable: lower 50 IU/mL. [d] Information was not found in patients' medical records. [e] Alcohol abuse was considered as more than 40 g per day for females and over 80 g per day for males.

TABLE 2: Allelic and genotypic frequencies for HPA-1 in patients with chronic hepatitis C with hepatocellular carcinoma (HCC) or without HCC and with lower fibrosis degree (F1 or F2)* (P<0.05)

	Patients with chronic hepatitis C and HCC (n=76)	Patients with chronic hepatitis C without HCC and with lower fibrosis degree (F1 or F2) (n = 81)**	*p-value*
Alleles			
HPA-1a	126	145	0.08
HPA-1b	26	17	
Genotypes			
HPA-1a/1a	53#	69#	
HPA-1a/1b	20#	07#	0.013
HPA-1b/1b	03	05	

HPA: Human Platelet Antigen; **HCV:** Hepatitis C virus. *According METAVIR Score. **Data reported by Silva et al. (2012). **#** significant difference according Fisher's Exact Test.

TABLE 3: Logistic regression of risk factors associated with HCC presence.

Variable	Odds Ratio (95% CI)
HPA 1a/1a vs. 1a/1b	0.2711 (0.0899 - 0.7291)*
HPA 1a/1a vs. 1b/1b	1.2778 (0.2364 – 8.5964)

Model adjusted considering hepatocellular carcinoma presence as response variable and HPA-1 genotype as risk factors. *significant difference according Fisher's Exact Test.

occurring after cirrhosis (F4), HCC presence has been observed in patients without cirrhosis[16].

Other factors which can be associated with HCC presence include genetic polymorphisms[3,4,5]. This study indicates that HPA-1 polymorphism is not associated with HCC presence (data not shown) in relation to HCV-infected patients without carcinoma.

On the other hand, the frequency of the HPA-1a/1b polymorphism was higher in HCV-infected patients with HCC than in those without HCC and with moderate fibrosis (F1/F2) (**Table 2**). This result suggests that HPA-1 system polymorphism may be more associated with liver damage progression than with the presence of HCC.

This result is in accordance with the HPA-1 frequencies described by Silva et al. (2011)[11], where the HPA-1a/1b polymorphism was associated with advanced fibrosis (F3/F4). In both situations (HCC and advanced fibrosis), the hepatic function is already greatly reduced, which is a characteristic of compromised liver function.

In conclusion, these data contribute to the understanding of the dynamics of HCV infection during HCC. In addition, the HPA-1a/1b polymorphism may represent a molecular biomarker for poor prognosis and higher hepatic damage in chronic hepatitis C.

Conflict of Interest

The authors declare that there is no conflict of interest.

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