

Genetic Variability of Hepatitis B and C Viruses in Brazilian Patients With and Without Hepatocellular Carcinoma

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Most cases of hepatocellular carcinoma (HCC) are due to chronic hepatitis B virus (HBV) or hepatitis C virus (HCV) infection worldwide. The aim of this study was to determine the viral genotypes and frequency of 17 mutations (15 for HBV and 2 for HCV), described previously as able to influence the course of chronic liver disease, in patients with and without HCC. This transversal study included 157 Brazilian patients with chronic hepatitis B ($n = 51$) and C ($n = 106$). Of these, 12 and 40 patients had HBV- and HCV-related HCC, respectively. Nucleotide sequencing of core promoter, pre-core, and pre-S/S regions of HBV and core region of HCV strains was performed to determine their genotypes and the frequency of the respective mutations. Among the HBV isolates, subgenotype A1 was the most prevalent in both patients with (90%) and without (61%) HCC. Fourteen out of the 15 mutations under study, as well as five different pre-S deletions, were identified. Core promoter T1753V, A1762T, and G1764A mutations were more frequent in patients with HCC than in those without, although with no statistical difference. However, a significant correlation was observed between T1753V mutation and elevation of transaminases levels ($P < 0.05$). As for HCV, mutation at residue 70 in the core protein of genotype 1b strains was significantly more frequent in patients with cirrhosis (56.3%) than in those without (9.1%) ($P = 0.018$). The detection of some key mutations in the genomes of HBV and HCV might be helpful to predict the clinical outcome of patients with chronic liver disease. **J. Med. Virol.** 86:217–223, 2014.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is a leading cause of cancer-related death worldwide, and the burden of this cancer is expected to increase further in coming years [Ferlay et al., 2008; Venook et al., 2010]. Up to 80% of HCC cases are associated with chronic hepatitis B virus (HBV) or hepatitis C virus (HCV) infection [Perz et al., 2006]. However, the precise pathogenetic mechanisms linking viral infection and HCC remain obscure.

Some virus genotypes and genetic mutations have been suggested to contribute to the severity of liver disease and increased risk of HCC [Tsai and Chung, 2010; El-Serag, 2012; Arzumanyan et al., 2013]. In a specific manner, HBV genotypes A1 [Kew et al., 2005], C [Kao et al., 2002; Chan et al., 2004; Chen et al., 2004] and F [Livingston et al., 2007], as well as HCV genotypes 1b [Bruno et al., 2007; Raimondi et al., 2009], and 3a [Idrees et al., 2009] have been found to be associated with higher rates of HCC development.

Nucleotide substitutions in the HBV core promoter (nts 1613–1849), resulting in a decreased expression of HBeAg but enhanced viral replication, as well as deletions in the pre-S region (leading to truncated pre-S/S proteins), have been associated with the progression of liver disease and HCC [Asim et al., 2010; Tsai and Chung, 2010; Kao et al., 2012; Kitab et al., 2012; Qu et al., 2013]. On the other side, the G1896A mutation in the pre-core region (nts

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1814–1900) of the HBV genome, which creates a premature stop codon, abolishing the production of HBeAg, has been associated with a decreased incidence of HCC [Liu et al., 2006; Yang et al., 2008].

HCV core protein alters host gene expression and binds to several tumor suppressor proteins [Tsai and Chung, 2010]. Several clinical studies, especially in Japanese populations, have revealed that mutations at core codons 70 and 91 of the HCV genotype 1b isolates were associated with HCC [Akuta et al., 2007; Kobayashi et al., 2010; Nakamoto et al., 2010]. However, other investigators did not find such an association for the mutation at position 91 [Fishman et al., 2009; El-Shamy et al., 2013].

In Brazil, approximately 9,000 new cases of HCC (2.9% of all cancers) are registered each year, and 5% of the cancer-related deaths are attributed to HCC [Ferlay et al., 2008]. However, no report has been published so far on the viral factors associated to HCC in Brazilian patients.

In this study, the viral genotypes and the presence of mutations in the core promoter, pre-core, and pre-S regions of HBV isolates, and in the core region of HCV strains, were investigated, and their frequencies compared in patients with and without HCC.

PATIENTS AND METHODS

Patients

The study included 51 HBsAg positive patients (12 with HCC and 39 with chronic liver disease but without HCC) and 106 HCV RNA positive patients (40 with HCC and 66 with chronic liver disease but without HCC) referred to the Clementino Fraga Filho University Hospital (HUCFF), Rio de Janeiro, Brazil, from April 2010 to March 2013. Demographic, laboratory, and clinical information, including age, gender, HBV serological status, liver function tests, and antiviral treatment were abstracted from the medical records of participants. HCV carriers without HCC were classified further into two groups, with and without cirrhosis. The diagnosis of liver cirrhosis was made by liver biopsy or by the presence of clinical and laboratory features of portal hypertension at ultrasound or upper endoscopy. HCC diagnosis was based on elevated serum alpha-fetoprotein level (>400 ng/ml) combined with at least one positive image on the angiography, ultrasonography, and/or computed tomography. Patients with normal levels of alpha-fetoprotein should have two image methods showing positive results for the diagnosis of HCC. The hospital's Ethics Committee approved the study protocol and an informed consent form was obtained from all patients.

Nucleic Acids Extraction and Amplification

HBV DNA and HCV RNA were extracted from 0.2 ml of serum by using High Pure Viral Nucleic

Acid kit (Roche Diagnostics, Mannheim, Germany) and resuspended in 50 μ l of nuclease free water.

HBV pre-S/S region was amplified by semi-nested polymerase chain reaction (PCR). The first round of amplification was performed with 4 μ l of DNA, 1 U of Taq DNA polymerase (Invitrogen, Carlsbad, CA), and sense PS1 (5'-CCATATTCTTGGGAACAAGA-3', nts 2826–2845) and antisense P3 (5'-AAAGCCCAAAA-GACCCACAA-3', nts 1000–1019) oligonucleotide primers. Second round PCR was performed with 2 μ l of the first round product, 1 U of Taq DNA polymerase (Invitrogen), sense PS1, and antisense S2 (5'-GGGTTTAAATGTATACCCAAAGA-3', nts 819–841) primers. Both rounds were performed in the same conditions, that is, 94°C for 3 min, 35 cycles at 95°C for 30 sec, 52°C for 40 sec, and 72°C for 2 min, followed by a final elongation at 72°C for 7 min. Core promoter and pre-C regions were amplified in a nested PCR assay carried out with 4 μ l of DNA, 1 U of Taq DNA polymerase (Invitrogen), sense X1 (5'-ACCTCCTTTCCATGGCTGCT-3', nts 1363–1382), and antisense C2 (5'-CTAACATTGAGATTCCCAGATTGAGA-3', nts 2432–2458) primers. The second round of amplification was performed using 2 μ l of the first round PCR product, 1 U of Taq DNA polymerase (Invitrogen), sense X4 (5'-AAGTCTTACATAAGAGGAC-3', nts 1644–1663), and antisense C3 (5'-TTGCCTGAGTGCAGTATGGT-3', nts 2056–2075) primers. PCR conditions for both rounds were 94°C for 2 min, 35 cycles at 94°C for 30 sec, 52°C for 30 sec, and 72°C for 1 min 30 sec, followed by a final elongation at 72°C for 7 min.

The HCV core region was amplified by reverse transcription (RT)-PCR. RT and the first round of PCR were performed in a single tube using the SuperScript III One-Step RT-PCR System with Platinum Taq kit (Invitrogen), 4 μ l of RNA, and outer primers Sc2 and Ac2 [Ohno et al., 1997]. RT step was for 45°C for 30 min, and was followed by DNA denaturation at 94°C for 2 min, 20 PCR cycles at 94°C for 1 min, 45°C for 1 min, and 68°C for 1 min, and 20 additional cycles at 94°C for 1 min, 60°C for 1 min, and 68°C for 1 min, and a final elongation at 68°C for 5 min. Second round PCR was carried out using 2 μ l of the first round PCR product, 1 U of Taq DNA polymerase (Invitrogen), and inner primers S7 and A5 [Ohno et al., 1997], under the following conditions: 94°C for 2 min; 30 cycles at 94°C for 1 min, 62°C for 1 min, and 72°C for 1 min, with a final extension step at 72°C for 7 min.

Direct Nucleotide Sequencing and Genotyping

PCR products were purified using the Wizard SV Gel and PCR Clean-Up System (Promega, Madison, WI) and bidirectionally sequenced using BigDye Terminator kit (Life Technologies, Carlsbad, CA). Sequencing reactions were analyzed on an ABI3730 automated sequencer (Life Technologies). HBV and HCV genotypes were determined by direct sequencing

of the pre-S/S and core amplicons, respectively, followed by phylogenetic analysis using MEGA software version 5.1 [Tamura et al., 2011].

Statistical Analysis

Statistical analyses were performed using Pearson chi-square test and Fisher's exact test for categorical variables, and Mann-Whitney's *U*-test or independent samples *t*-test for quantitative variables, as appropriate. Differences were considered significant for *P* values less than 0.05. The software used was the statistical analysis SPSS software, version 18.0.

RESULTS

Characteristics of Patients Infected With HBV and HCV

Demographic, biochemical, and serological characteristics of the 51 HBV chronic carriers of this study are shown in Table I. The mean age of patients with HCC was significantly higher than in patients without HCC (58.8 ± 10.3 vs. 40.4 ± 16.4 years, $P < 0.001$). A major proportion of male was observed in the HCC group (83.3% vs. 66.7%). Also, HCC group had significantly higher aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (GGT), and total bilirubin (T.BIL) levels, as well as lower serum albumin (ALB) values than the group without HCC ($P < 0.05$). HBeAg positivity was observed in 2/12 (16.7%) patients with HCC and 14/39 (35.9%) patients without HCC. Fifty percent of the patients with HCC were under antiviral treatment, compared to 25.6% of those without HCC.

TABLE I. Baseline Characteristics of HBV Infected Patients

Characteristics	HCC (n = 12)	Without HCC (n = 39)	<i>P</i> -Value
Age (years, mean \pm SD)	58.8 ± 10.3	40.4 ± 16.4	< 0.001
Male (%)	10 (83.3%)	26 (66.7%)	NS
ALT (U/L, mean \pm SD)	53.2 ± 30.0	54.3 ± 42.7	NS
AST (U/L, mean \pm SD)	84.3 ± 74.5	42.9 ± 30.0	0.006
ALP (U/L, mean \pm SD)	261.3 ± 273.6	99.6 ± 78.7	0.003
GGT (U/L, mean \pm SD)	167.3 ± 139.4	57.5 ± 34.6	0.012
ALB (g/dl, mean \pm SD)	3.5 ± 0.8	4.2 ± 0.6	0.010
T.BIL (mg/dl, mean \pm SD)	1.7 ± 1.4	0.5 ± 0.3	< 0.001
HBeAg positive (%)	2 (16.7%)	14 (35.9%)	NS
Antiviral treatment (%)	6 (50.0%)	10 (25.6%)	—

HCC, hepatocellular carcinoma; SD, standard deviation; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyl transpeptidase; ALB, serum albumin; T.BIL, total bilirubin; NS, not significant.

Table II shows the demographic and biochemical characteristics of the 106 HCV infected patients, classified in HCC and without HCC carriers. The group of patients without HCC was divided further into cirrhosis and without cirrhosis subgroups. The mean age of the HCC patients was significantly higher than those with ($P = 0.047$) and without cirrhosis ($P = 0.002$). The group without cirrhosis had a lower proportion of male compared to the HCC ($P = 0.008$) and cirrhosis ($P = 0.011$) groups, respectively. No statistically significant difference was observed between HCC and cirrhosis groups in relation to ALT, AST, ALP, GGT, ALB, and T.BIL levels. However, the group without cirrhosis had significantly higher ALB values and lower ALT, AST, ALP, GGT, and T.BIL levels ($P < 0.05$ for all observations) than the other two groups.

Genotypes Distribution of and Key Mutations in the HBV Isolates

The distribution of HBV genotypes/subgenotypes and the occurrence of various mutations in the core promoter (including upper regulatory region and basal core promoter) and pre-core regions of the HBV isolates are shown in Table III. Subgenotype A1 was the most frequent in both HCC and without HCC groups, being found in 9 out of 10 patients with HCC. In the upper regulatory region of the core promoter, T1636R and C1637W were only found in patients without HCC, whereas C1653T was found in both HCC and without HCC groups. Substitutions T1753V, A1757G, A1762T, G1764A, C1766K, and T1768A, in the basal core promoter, were also detected in both groups. A1762T was present in association with G1764A in 50.0% and 33.3% of patients with and without HCC, respectively. None of the five pre-core mutations under study was present in patients with HCC. However, substitutions A1814Y, T1815M, G1896A, and G1899A were found in 3–15% of the patients without HCC. The combination (i) double mutant at nts 1762 and 1764 and (ii) wild-type at nt 1896 was found in isolates infecting both patients with and without HCC, although with a higher frequency among HCC patients (50.0% vs. 25.9%).

The presence of pre-S deletions in the HBV genome was observed in 1/8 (12.5%) and 4/34 (11.8%) patients with and without HCC, respectively. Four deletions occurred in the pre-S2 region and one in the pre-S1 region. Their length was always a multiple of 3 nucleotides, and varied from 6 to 72 nucleotides (Table IV).

Genotypes Distribution of the HCV Isolates and Mutations in the Core Protein

Table V shows the genotypes distribution of the 106 HCV isolates of this study, as well as the frequency of mutations in the core protein at residues 70 (R70Q) and 91 (L91M) (only genotype 1b isolates

TABLE II. Baseline Characteristics of HCV Infected Patients

Characteristics	Without HCC			P-Value			
	HCC (n = 40)	Cirrhosis (n = 40)	Without cirrhosis (n = 26)	HCC vs. without HCC	HCC vs. without cirrhosis	Cirrhosis vs. without cirrhosis	HCC + cirrhosis vs. without cirrhosis
Age (years, mean ± SD)	63.6 ± 7.9	59.7 ± 10.3	56.1 ± 8.5	0.004	0.047	NS	0.011
Male (%)	23 (57.5%)	23 (56.1%)	6 (24.0%)	NS	NS	0.011	0.004
ALT (U/L, mean ± SD)	90.4 ± 40.6	126.4 ± 103.7	77.1 ± 47.1	NS	NS	0.031	0.013
AST (U/L, mean ± SD)	110.8 ± 52.7	126.6 ± 124.1	62.4 ± 48.7	0.012	NS	0.004	<0.001
ALP (U/L, mean ± SD)	186.7 ± 143.8	128.3 ± 54.7	76 ± 28.5	0.010	NS	<0.001	<0.001
GGT (U/L, mean ± SD)	202.8 ± 206.4	155.6 ± 139.2	72.3 ± 32.3	0.048	NS	0.010	0.002
ALB (g/dl, mean ± SD)	3.7 ± 0.6	3.4 ± 0.8	4.0 ± 0.3	NS	NS	<0.001	<0.001
T.BIL (mg/dl, mean ± SD)	1.2 ± 0.7	1.7 ± 2.9	0.8 ± 0.8	NS	NS	0.005	0.001
Antiviral treatment	18 (52.9%)	21 (67.7%)	17 (70.8%)	—	—	—	—

HCC, hepatocellular carcinoma; SD, standard deviation; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyl transpeptidase; ALB, serum albumin; T.BIL, total bilirubin.

TABLE III. HBV Genotypes Distribution and Mutations in the HBV Genome

Genotypes and mutations	Number of isolates (%)	
	HCC	Without HCC
Genotype/subgenotype	n = 10	n = 36
A1	9 (90.0)	22 (61.1)
A2	1 (10.0)	6 (16.7)
D1	0 (0)	1 (2.8)
D6	0 (0)	2 (5.5)
D7	0 (0)	1 (2.8)
F2	0 (0)	2 (5.5)
F4	0 (0)	1 (2.8)
F4 and G coinfection	0 (0)	1 (2.8)
Core promoter		
Upper regulatory region	n = 5	n = 17
T1636R	0 (0)	4 (23.5)
C1637W	0 (0)	4 (23.5)
C1653T	1 (20.0)	3 (17.6)
Basal core promoter	n = 6	n = 27
T1753V	2 (33.3)	6 (22.2)
A1757G	5 (83.3)	26 (96.3)
A1762T	4 (66.7)	10 (37.0)
G1764A	3 (50.0)	11 (40.7)
C1766K	1 (16.7)	5 (18.5)
T1768A	1 (16.7)	4 (14.8)
A1762T/G1764A	3 (50.0)	9 (33.3)
Pre-core	n = 6	n = 27
A1814Y	0 (0)	3 (11.1)
T1815M	0 (0)	1 (3.7)
G1816T	0 (0)	0 (0)
G1896A	0 (0)	4 (14.8)
G1899A	0 (0)	2 (7.4)
Core promoter-pre-core combinations	n = 6	n = 27
1762/1764 wild-1896 wild	2 (33.3)	13 (48.1)
1762/1764 mut-1896 wild	3 (50.0)	7 (25.9)

for L91M). Genotypes 1a, 1b, and 3a were the only ones found. In all three groups of patients (HCC, cirrhosis and without cirrhosis), genotypes 1a (34–50%) and 1b (35–42%) were more frequent than 3a (10–23%). Amongst patients infected with HCV genotype 1b, the mutation R70Q was significantly more frequent in patients with cirrhosis than in those without (56.3% vs. 9.1%, $P=0.018$) and in HCC plus cirrhosis groups than without cirrhosis group (50.0% vs. 9.1%, $P=0.028$). However, no significant association was observed for mutation L91M, for which similar frequencies were obtained in the three groups of patients.

Association Between HBV T1753V Mutation and Age and Transaminases Levels of the Patients

Table VI shows the mean age and transaminases levels of the HBV infected patients and their association with the presence of determined mutations on the viral genome. Patients infected with a virus isolate (i) from subgenotype A1, (ii) showing a deletion in the pre-S region, or (iii) with one of the mutations C1653T, T1753V, A1762T, or G1764A, had higher mean levels of ALT and AST than the others. However, this difference of transaminase levels was

TABLE IV. Deletions in the Pre-S Region of HBV Isolates

Patient		HBV isolate			
Liver disease	Sex/age (years)	Subgenotype	Region	Length (bp)	Segment (nt) ^a
HCC	M/63	A1	Pre-S2	48	378–425
Without HCC	F/63	A1	Pre-S2	6	417–422
Without HCC	M/39	A1	Pre-S2	51	370–420
Without HCC	M/29	A2	Pre-S2	6	421–426
Without HCC	M/29	D6	Pre-S1	72	197–268

^aNumbering starts from the initiation codon of pre-S1 region and is based on a genotype A isolate (complete genome = 3,221 bp).

only statistically significant ($P < 0.05$) in the case of the T1753V mutation. In addition, patients infected with HBV mutated at nt 1753 were significantly older than the others ($P = 0.002$).

In a similar manner, data related to mean age and transaminases levels of the HCV infected patients were computed in an attempt to correlate them with virus genotype and presence of R70Q and L91M mutations. However, no association was observed between these parameters (not shown).

DISCUSSION

A recent Brazilian national survey has shown that HBV and HCV infections accounted for 16% and 54% of the cases of HCC, respectively [Carrilho et al., 2010]. The goal of this study was to assess the genotype distributions of the HBV and HCV isolates infecting Brazilian patients with and without HCC as well as to evaluate possible relationships between viral mutations and severity of liver disease. HBV strains from subgenotypes A1, A2, D1, D6, D7, F2, and F4 were detected. Overall frequencies of genotypes A, D, and F were 84.4%, 8.8%, and 6.7%, respectively, in accordance to previous phylogenetic studies showing that genotypes A, D, and F were the most prevalent in Brazil [Araujo et al., 2004; Mello et al., 2007; Motta-Castro et al., 2008]. One case of coinfection with HBV isolates from genotypes F4 and

G was detected (in a patient without HCC), and complete nucleotide sequences of the two genotypes were published elsewhere [Araujo et al., 2013]. Nine out of 10 of the isolates infecting HBV-related HCC patients were from subgenotype A1 (Table III). It is noteworthy that subgenotype A1 has been associated with very high rates of HCC in sub-Saharan Africa [Kramvis et al., 1998; Kew et al., 2005]. A case-control study of HBsAg-positive African patients has found a 4.5-fold increased risk of HCC in those infected with subgenotype A1 versus other genotypes [Kew et al., 2005]. Conversely, the rate of complications, including HCC, in patients infected with subgenotype A2 has been shown to be lower than in those infected with genotypes C, D, or F1 [Sanchez-Tapias et al., 2002; Livingston et al., 2007].

A number of point mutations in the HBV core promoter and pre-core regions, including T1636R, C1637W, C1653T, T1753V, A1757G, A1762T, G1764A, C1766K, T1768A, A1814Y, T1815M, G1816T, G1896A, and G1899A, as well as in-phase deletions in the pre-S region, have become increasingly recognized to be associated with severe clinical outcome and HCC development [Asim et al., 2010; Tsai and Chung, 2010; Kao et al., 2012; Qu et al., 2013]. All of these mutations, with the exception of G1816T, were detected in this study. Although some mutations were more frequent among patients with HCC than in the others, the differences were not statistically significant.

TABLE V. Genotypes Distribution of the HCV Isolates and Mutations in the Core Protein

Genotypes and mutations	HCC (n = 40)	Without HCC		P-Value
		Cirrhosis (n = 40)	Without cirrhosis (n = 26)	
Genotype				
1a	17 (42.5%)	20 (50.0)	9 (34.6)	NS
1b	14 (35.0%)	16 (40.0)	11 (42.3)	NS
3a	9 (22.5%)	4 (10.0)	6 (23.1)	NS
Mutation R70Q				
1a	2/17 (11.8%)	0/20 (0%)	1/9 (11.1%)	NS
1b	6/14 (42.9%)	9/16 (56.3%)	1/11 (9.1%)	<0.05 ^a
3a	2/9 (22.2%)	0/4 (0%)	2/6 (33.3%)	NS
Mutation L91M				
1b	11/14 (78.6%)	12/16 (75.0%)	8/11 (72.7%)	NS

^aSignificant P values were obtained when comparing cirrhosis versus without cirrhosis ($P = 0.018$) and HCC + cirrhosis versus without cirrhosis ($P = 0.028$) groups of patients. Other comparisons gave no significant results (NS).

TABLE VI. Mean Age and Transaminase Levels of the HBV Infected Patients According to Virus Genotypes and Particular Mutations

Genotypes/mutations	Age (years)		ALT (U/L)		AST (U/L)	
	Mean \pm SD	<i>P</i> -Value	Mean \pm SD	<i>P</i> -Value	Mean \pm SD	<i>P</i> -Value
Subgenotype						
A1	45.6 \pm 16.6	NS	49.1 \pm 30.5	NS	54.3 \pm 57.5	NS
A2	42.1 \pm 16.7		28.2 \pm 14.8		36.0 \pm 21.9	
Pre-S deletion						
Presence	44.6 \pm 17.3	NS	87.1 \pm 60.7	NS	61.6 \pm 50.0	NS
Absence	43.7 \pm 15.8		48.6 \pm 35.2		52.1 \pm 53.0	
C1653T						
Presence	47.0 \pm 15.7	NS	71.9 \pm 51.0	NS	80.0 \pm 51.1	NS
Absence	49.3 \pm 16.2		60.7 \pm 39.5		68.5 \pm 74.7	
T1753V						
Presence	56.6 \pm 13.2	0.002	73.5 \pm 39.8	0.036	91.4 \pm 75.7	0.005
Absence	37.3 \pm 16.3		46.3 \pm 37.1		39.6 \pm 27.5	
A1762T						
Presence	46.0 \pm 18.3	NS	65.1 \pm 39.0	NS	68.1 \pm 37.1	NS
Absence	43.2 \pm 17.5		50.6 \pm 40.3		45.3 \pm 29.5	
G1764A						
Presence	44.2 \pm 16.7	NS	58.3 \pm 38.3	NS	62.5 \pm 36.6	NS
Absence	44.3 \pm 18.8		54.3 \pm 42.0		46.9 \pm 30.7	
G1896A						
Presence	44.0 \pm 12.5	NS	55.7 \pm 53.1	NS	48.0 \pm 40.7	NS
Absence	44.9 \pm 18.2		56.6 \pm 38.6		65.1 \pm 60.0	

ALT, alanine aminotransferase; AST, aspartate aminotransferase; SD, standard deviation; NS, not significant.

However, a significant association was observed between mutation T1753V and elevation of ALT and AST levels (Table VI). Such an association has also been found in Moroccan HBV chronic carriers [Kitab et al., 2012].

All the HCV isolates infecting patients of this study were from genotypes 1a (43.0%), 1b (38.7%), or 3a (17.9%). Such a genotype distribution was consistent with previous studies performed with Brazilian samples describing frequencies of 64–87%, 2–5%, 13–30%, <1%, and <1% for genotypes 1–5, respectively [Martins et al., 1998; Oliveira et al., 1999; de Almeida et al., 2004; Campioto et al., 2005].

The relative risk for HCC has been estimated to be 2.5-fold higher among patients infected with HCV genotype 1b than with other genotypes [Raimondi et al., 2009]. Several studies have suggested that a mutation at residue 70 in the core protein of genotype 1b isolates was associated with HCC development [Akuta et al., 2007; Fishman et al., 2009; Khaliq et al., 2011; El-Shamy et al., 2013]. In this respect, a possible role of mutation L91M has also been mentioned [Akuta et al., 2007; Khaliq et al., 2011]. The core protein with mutations at residues 70 and 91 has been shown to bind and activate protein kinase R, which might cause carcinogenesis [Delhem et al., 2001]. Another study has shown that non-wild-type 70/91 pattern was significantly associated with cirrhosis development in patients with F0–F3 fibrosis [Nakamoto et al., 2010]. Moreover, it has been demonstrated that the mutation at codon 70 was associated with more advanced liver fibrosis stage in Taiwanese HCV genotype 1 patients [Hsu et al., 2011]. In this study, mutation at codon 70 in

HCV genotype 1b strains was significantly more frequent in patients with cirrhosis than in those without (56.3% vs. 9.1%, $P=0.018$) and in HCC plus cirrhosis groups than without cirrhosis group (50.0% vs. 9.1%, $P=0.028$; Table V). This constituted one more line of evidence suggesting that a mutation at HCV core residue 70 may facilitate HCC development by accelerating fibrosis and/or cirrhosis.

To our knowledge, this is the first study that assessed the genetic variability of HBV and HCV isolates among Brazilian HCC patients. Further studies are necessary to determine whether given genotypes and mutations in the genomes of HBV and HCV might be used as biomarkers leading to improved early detection strategies and more effective therapies for patients with HBV- or HCV-related HCC.

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