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Distribution of HCV genotypes among different exposure categories in Brazil

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Abstract

Hepatitis C virus (HCV) infection is widespread and responsible for more than 60% of chronic hepatitis cases. HCV presents a genetic variability which has led to viral classification into at least 6 genotypes and a series of subtypes. These variants present characteristic geographical distribution, but their association with different responses to treatment with interferon and severity of disease still remains controversial. The aim of this study was to investigate the patterns of distribution of HCV genotypes among different exposure categories in Brazil. Two hundred and fifty anti-HCV positive samples were submitted to HCV-RNA detection by RT-PCR and their genotype was determined by restriction fragment length polymorphism (RFLP) analysis. In addition, the genotype/subtype of 60 samples was also determined by a reverse hybridization assay. HCV 1 was the most prevalent (72.0%), followed by type 3 (25.3%), HCV 2 (2.0%) and HCV 4 (0.7%). The HCV genotype distribution varied among the different exposure categories, with HCV 1 being more frequent among blood donors, hemophiliacs and hemodialysis patients. A high frequency of HCV 3 was observed in cirrhotic patients, blood donors from the South of Brazil and injecting drug users (IDUs). The general distribution of the HCV genotype in Brazil is similar to that in other regions of the world.

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Hepatitis C virus (HCV) was identified by Choo et al. (1) and there is a growing consensus to classify it into the family *Flaviviridae* (2). Hepatitis C constitutes a public health issue of major importance since more than 90% of patients develop chronic liver disease and this viral infection is considered to be the major risk factor for hepatocellular carcinoma in the world (3,4).

The HCV genome consists of a positive stranded RNA of approximately 9.5 kb. The genetic heterogeneity of HCV led to its classification into phylogenetic clusters. The widest variation is observed among HCV isolates of distinct genotypes, which present >25% of nucleotide divergence within the entire genome or subgenomic parts. These genotypes are further divided into subtypes, which present about 75-86% nucleotide homology. The narrowest divergence is found between viruses from a single strain, which constitute a quasispecies. Although there is no standardized classification system for HCV genetic variants, most investigators agree that there are at least 6 major genotypes and a series of subtypes (2,5).

Epidemiological data suggest that the distribution of HCV genotypes varies among different regions (3,5,6). In addition, an association between the genetic variants and routes of transmission has been reported (7,8). Although some authors found a correlation of HCV genotypes with clinical response to interferon therapy (9) as well as with the outcome of the disease (10), these aspects still remain controversial (11,12).

Little is known about the molecular epidemiology of HCV in Brazil. The aim of the present study was to investigate the distribution of HCV genotypes among Brazilian subjects according to their exposure categories.

Sera and epidemiological data were kindly supplied by different public health laboratories, public blood banks and other medical institutions from different Brazilian states, from 1994 to 1996. A total of 250 anti-HCV-positive samples were investigated for the presence of HCV-RNA by RT-PCR.

Among the 78 blood donors tested, 8 samples were from the State of Acre (AC), 14 from Amazonas (AM), 9 from Rio Grande do Sul (RS), 24 from Pará (PA) and 23 from Rio de Janeiro (RJ). In addition, we tested 13 samples from cirrhotic patients from the State of Espírito Santo (ES), 30 samples from hemophiliacs and 56 from injecting drug users (IDUs) from Rio de Janeiro. We also tested 73 samples from hemodialysis patients from different states: 22 from Ceará (CE), 18 from São Paulo (SP), 14 from Minas Gerais (MG) and 19 from Mato Grosso (MT).

Viral RNA was extracted from 200 µl of plasma, using the QIAmp Blood kit and QIAmp Tissue kit (QIAGEN GmbH, Hilden, Germany). The extracted RNA was eluted in 100 µl of DEPC-treated water. Reverse transcription (RT) was carried out with 2.5 µl of RNA in a final volume of 10 µl. The mixture was submitted to one cycle of 42°C/60 min and another of 95°C/5 min. In the first and second PCR rounds we targeted sequences within the HCV 5' non-coding region (HCV 5'NCR) and we used 2.5 µl of RT and first PCR product, respectively, in a final volume of 25 µl. The cycling protocol included 35 cycles of 94°C/1 min, 54°C/1 min and 72°C/2 min, followed by a final cycle of 72°C/5 min. The sequences and genomic positions for the outer primers used were 1b (5'GGTG CACGGTCTACGAGACC3'; 251-231) and 2a (5'GGCGACACTCCRCGAT3'; 5-21) and the inner primers were 56 (5'CGCAAGCA CCCTATCAGGGCAGT3'; 35-21) and 4 (5'GAGGAACTACTGTCTTCACGCAGAA3'; 218-195). The 260-bp PCR product was submitted to electrophoresis using a 2% agarose gel in TBE buffer and visualized by ethidium bromide staining under ultraviolet light.

HCV genotyping by restriction fragment length polymorphism analysis (RFLP) was performed as described elsewhere (8) and genotypes were determined according to Simmonds' classification (13). Since RFLP did not permit the determination of HCV subtypes, 60 samples previously genotyped by RFLP were also submitted to reverse hybridization (RH) using the INNOLIPA HCV II test kit (Innogenetics, Zwijndrecht, Belgium).

Univariate analyses, comparing HCV genotypes distribution between different exposure categories, were carried out using Epiinfo version 5.0 (Centers for Disease Control and Prevention, Atlanta, GA, USA, 1990). The chi-square or 2-tailed Fisher exact test was employed and results were considered to be statistically significant when $P<0.05$.

One hundred and sixty (64%) of the 250 anti-HCV-positive samples were also positive for HCV RNA. Table 1 shows that the predictive value of PCR was higher among persons at risk such as hemophiliacs (100%), IDUs (76.8%), and cirrhotic patients (76.7%). The low frequency of HCV-RNA found among blood donors from the northern states may have been a consequence of sample damage due to unsuitable conditions of transportation and/or storage. HCV genotype was determined by RFLP in 150 samples. The most

Table 1 - Distribution of HCV genotypes among different exposure categories.

AC, Acre; AM, Amazonas; PA, Pará; RJ, Rio de Janeiro; RS, Rio Grande do Sul; ES, Espírito Santo; CE, Ceará; MT, Mato Grosso; MG, Minas Gerais; SP, São Paulo. *P<0.04 (compared with blood donors and hemophiliacs from Rio de Janeiro (χ^2 or Fisher exact test).

Exposure category	N tested	%PCR positive	HCV genotypes				Total
			1 (N, %)	2 (N, %)	3 (N, %)	4 (N, %)	
Blood donors	78	50.0	27 (79.4)	-	7 (20.6)	-	34
AC	8	25.0	2 (100.0)	-	-	-	2
AM	14	7.1	1 (100.0)	-	-	-	1
PA	24	41.6	9 (90.0)	-	1 (10.0)	-	10
RJ	23	78.2	13 (92.8)	-	1 (7.2)	-	14
RS	9	88.8	2 (28.5)	-	5 (71.5)	-	7
Cirrhotic patients (ES)	13	76.9	6 (60.0)	-	4 (40.0)	-	10
Hemophiliacs (RJ)	30	100.0	24 (80.0)	-	5 (16.6)	1 (3.4)	30
Injectable drug users (RJ)	56	76.8	22 (56.4)	3 (7.7)	14 (35.9)*	-	39
Patients under hemodialysis	73	52.0	29 (78.4)	-	8 (21.6)	-	37
CE	22	45.4	5 (50.0)	-	5 (50.0)	-	10
MT	19	52.6	10 (100.0)	-	-	-	10
MG	14	57.1	8 (100.0)	-	-	-	8
SP	18	55.5	6 (66.6)	-	3 (33.4)	-	9
Total	250	64.0	108 (72.0)	3 (2.0)	38 (25.3)	1 (0.7)	150

prevalent genotype was HCV 1 (109/150, 72.7%), followed by HCV 3 (38/150, 25.3%) and HCV 2 (3/150, 2.0%).

In the 60 samples tested by RH, the most frequent subtype was 1b (35.0%), followed by 1a (30.0%), 3a (24.9%), 1a and 1b (5.0%) and subtypes 1 and 4c4d (1.7% each).

Except for one sample in which HCV 1 was identified by RFLP and HCV 4c4d was identified by RH, the two methods showed 98.3% agreement.

The distribution of HCV genotypes among the different exposure categories is presented in Table 1. Among blood donors, 79.4% (27/34) had HCV 1 and 20.6% (7/34) had HCV 3. Among subjects from the northern region of Brazil (AC, PA and AM) and Rio de Janeiro (southeast), we found a predominance of HCV 1 infection (92.3 and 92.8%, respectively). However, a different profile was observed in blood donors from RS (south), among whom genotype 3 was the most prevalent (71.5%).

HCV 1 was detected in 78.4% (29/37) of samples from hemodialysis patients whereas HCV 3 was detected in 21.6% (8/37). Interestingly, individuals from the State of Ceará showed a higher frequency of HCV 3 (50.0%) than subjects from other states. A high frequency of HCV 1 (80.0%) was found among hemophiliacs, the group in which the only HCV 4 case was found in this study. Among cirrhotic patients, the majority of subjects were infected with genotype 1a, followed by genotype 3.

Although the most prevalent HCV genotype among IDUs was HCV 1 (56.4%), a relatively high proportion of HCV 3 was noted in this population if compared with other exposure categories in Rio de Janeiro ($P = 0.04$). Among blood donors, HCV 1 was the most frequent genotype. Interestingly, a higher frequency of genotype 3 was observed in the south. These results partially conflict with previous findings (14) where HCV 1 was the most frequent, although the authors also reported a high prevalence of HCV type 3 in that region.

Among hemodialysis patients, a significantly higher prevalence of genotype 3 was

observed in subjects from Ceará and studies with larger number of samples should be carried out to confirm these findings.

Among hemophiliacs, genotype 1 was the most frequent. This has also been observed in Argentina (15) and the United States (5). The distribution of HCV genotypes among IDUs was similar to that observed in Berlin, Germany (16).

The agreement between the RFLP and INNOLIPA II results was 98.3%. RFLP is faster and easier to perform compared with other techniques, and suitable for molecular epidemiological studies of large number of samples.

The general distribution of HCV genotypes in different exposure categories in Brazil was similar to that for Europe, with a higher frequency of genotypes 1 and 3, followed by type 2 and sparse cases of type 4 (5). Future more extensive surveys should be conducted to assess geographic differences in distribution of HCV genotypes in Brazil.

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