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Hepatitis C virus genotypes in Southern Brazil

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Abstract

The prevalence of hepatitis C virus (HCV) genotypes in Southern Brazil was studied in the plasma of 100 HCV-RNA-positive patients attended in Porto Alegre, South of Brazil. Reverse transcription-polymerase chain reaction (RT-PCR) products from the 5' noncoding region were double digested with *Rsa*I-*Hae*III and *Bst*NI-*Hin*fI and analyzed by restriction fragment length polymorphism (RFLP). Three genotypes (1, 2 and 3) were demonstrable, the most prevalent being HCV type 1 (55 of 100 patients, 55%), followed by HCV type 3 (37 of 100 patients, 37%) and HCV type 2 (8 of 100 patients, 8%). There was an unusual high prevalence of genotype 3, in contrast to the majority of published data from the Southeast region.

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Hepatitis C virus (HCV) is the major etiological agent of non-A, non-B hepatitis. The virus has a positive-sense/RNA genome and is classified as a separate genus in the family Flaviviridae (1). The genome contains approximately 9,400 nucleotides and is organized into different regions: 5' noncoding region (5' NCR), core (C), envelope 1 (E1), envelope 2/non-structural protein 1 (E2/NS1), non-structural proteins 2-5 (NS2, NS3, NS4 and NS5) and 3' noncoding region (3' NCR) (2). Studies based on DNA sequencing have shown that different HCV isolates present a substantial nucleotide sequence variability distributed throughout the viral genome. Regions encoding the gene envelope proteins (E1, E2) are the most variable ones, whereas the 5' noncoding region is the most conserved one (3). Comparative sequence analysis of HCV genomes from different geographic regions has shown that HCV can be grouped into distinct but related genotypes (4). Currently, at least 9 major HCV genotypes and more than 20 subtypes have been identified by DNA sequencing (5).

The development of molecular methods that do not require the complex and time-consuming process of genome sequencing, such as type-specific reverse transcription-polymerase chain reaction (RT-PCR) amplification or hybridization (line probe assay, LIPA) (6,7) and restriction fragment length polymorphism (RFLP) of amplified fragments of the 5' NCR region (8), has made it

possible to characterize many HCV genomes throughout the world. Some of these results have demonstrated that the worldwide HCV genotype distribution varies according to geographical areas: types 1, 2 and 3 are found predominantly in Europe, Japan and the United States (5), type 4 in Central/North Africa and the Middle East (8), type 5 in South Africa (9), type 6 in Hong Kong (10) and types 7, 8 and 9 in Vietnam (11). In Brazil, recent studies performed in the Southeast region (States of São Paulo and Rio de Janeiro) demonstrated the presence of 3 HCV genotypes (1, 2 and 3) (Refs. 7,12,13).

The purpose of the present study was to determine the prevalence of HCV genotypes in Southern Brazil using RFLP of amplified fragments of the 5' NCR region, according to previous data (8).

One hundred consecutive HCV-RNA-positive plasma samples obtained in the period between August/95 and March/96 from the same number of patients were analyzed in a reference molecular biology laboratory (Simbios Biotecnologia, Porto Alegre, RS, Brazil). All samples were stored at -20°C and processed within a maximum of 7 days after collection. RNA was extracted from 100 µl of plasma by the guanidinium isothiocyanate-phenol-chloroform adapted method (14). Reverse transcription (RT) was carried out at 37°C for 30 min, using 75 mM KCl, 50 mM Tris-HCl, pH 8.3, 3 mM $MgCl_2$, 2.5 mM DTT, 1 mM dNTPs, 20 U of Moloney murine leukemia virus reverse transcriptase (Gibco BRL Life Technologies, USA), 8 U RNasin (Promega Corp., Madison, WI, USA), 2.0 µM antisense primer (5' CATGGTGCAC GGTCTACGAGACC 3') of the 5' NCR and 3 µl RNA. The amplification reaction was carried out using 10 µl cDNA, 10 mM Tris-HCl, pH 8.3, 1.5 mM $MgCl_2$, 50 mM KCl, 0.2 mM dNTPs, 2.0 µM of sense primer (5' GGCGACACTCCACCATAGATC 3') of the 5' NCR and 1.5 U *Taq* DNA polymerase (Centro de Biotecnologia, RS, Brazil). Amplification was performed for 35 cycles in an MJ Research PTC-100 thermal cycler, with cycling temperatures and times of 94°C for 30 sec, 50°C for 30 sec and 72°C for 60 sec. A final extension step of 72°C for 7 min was also included.

The products were separated by agarose gel electrophoresis and visualized by ethidium bromide staining. The size of the RT-PCR product was 325 bp. A nested RT-PCR with inner sense (5' GGAACTACTGT CTTCACGCAGA 3') and antisense (5' TCGCAAGCACCCTATCAGGCAG 3') primers was performed with 1 µl of the product from the second amplification to confirm the results. In addition, positive and negative samples were added as controls. HCV genotyping was performed according to the method described by McOmish et al. (8) using the external RT-PCR product for the restriction assay. Briefly, a 5-µl aliquot of the external RT-PCR product was digested with 1 U each of *Rsa*I and *Hae*III in the appropriate buffer for 1.5-2 h at 37°C, and with 1 U each of *Bst*NI *(Mva*I isoschizomer*)* and *Hin*fI in the appropriate buffer for 1.5 h at 37°C plus 1.5 h at 60°C. The digested product was separated by electrophoresis on a 12.5% polyacrylamide gel and visualized by rapid silver staining (15). The banding patterns for the different HCV genotypes were deduced from those previously described (8) and from the 5' NCR sequences obtained from a gene database.

Figure 1 illustrates the banding patterns obtained with both sets of enzymes. These patterns are typical for genotypes 1, 2 and 3, as described by McOmish et al. (8). The same genotypes were found in the Southeast region in studies using the LIPA technique (7,12,13). However, in our study no mixed infections were observed, in contrast to the 2 mixed infections (both genotype 1 and genotype 3) reported in one of the previous studies (12).

Of the 100 samples examined, 55 (55%) were identified as genotype 1, 8 (8%) as genotype 2 and 37 (37%) as genotype 3.

These results show that genotype 1 is the most prevalent in Southern Brazil. Genotype 1 has been considered to be the most prevalent in Brazil, occurring in more than 70% of the HCV-positive patients (7,12,13). However, our study has demonstrated a lower prevalence of genotype 1 and a higher prevalence of genotype 3. These contrasting data could be explained by the fact that previous studies were carried out in a different region (Southeast) and sometimes performed with restricted epidemiological groups (mainly hemodialysis and liver disease patients). It is well known that HCV genotype 1 is more prevalent among patients with chronic hepatitis than in asymptomatic patients (16). Probably, the incidence of HCV genotype 1 in Brazil is not as high as stated before. New studies should be performed to establish the real prevalence of HCV genotypes in our country, mainly in the Northeast and North regions.

Besides being classified into major genotypes, HCV can be divided into subgroups (or subtypes) (5). HCV subtyping can be performed by type-specific RT-PCR amplification (6), reverse hybridization (12) or RFLP of amplified fragments of the 5' NCR region (17). Although a recent study showed that different subtypes from the same genotype usually respond equally to therapy (18), it is still important to determine the prevalent HCV subtypes. Studies are in progress to identify the prevalent HCV subtypes in Southern Brazil.

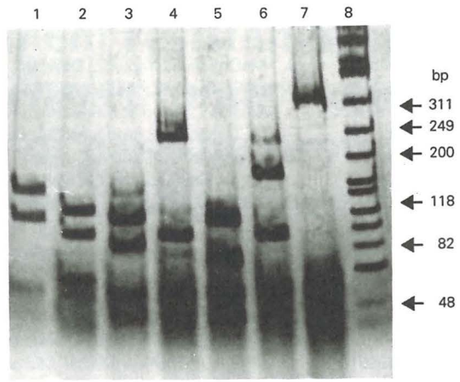

Figure 1 - Genotyping of HCV by RFLP analysis. RT-PCR products were electrophoresed on a 12.5% polyacrylamide gel and visualized by rapid silver staining. HCV type 1: lanes 1 and 2; type 2: lanes 3 and 4, and type 3: lanes 5 and 6. *Lanes 1, 3 and 5*: *Rsa*I-*Hae*III double digests of the 5' NCR PCR product; *lanes 2, 4 and 6*: *Bst*NI-*Hin*fI double digests of the 5' NCR PCR product; *lane 7*: 5' NCR PCR product not digested; *lane 8*: ϕX174 DNA-*Hin*fI weight marker.

The determination of HCV genotypes and subtypes present in Brazil is very important for epidemiological surveillance, blood donor screening and adequacy of foreign serological tests. Furthermore, the demonstration that infections with HCV genotypes 1 and 2 result in different responses to treatment (19,20) supports the importance of HCV genotyping in clinical settings. However, few data are reported concerning the treatment response of HCV genotype 3-positive patients. With the increasing incidence of genotype 3 in Brazil, studies should be conducted to determine the liver disease progression and treatment response of these patients.

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