BRIEF COMMUNICATION

HEPATITIS C VIRUS GENOTYPES IN HEMODIALYSIS PATIENTS IN THE FEDERAL DISTRICT, BRAZIL

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SUMMARY

Hepatitis C virus (HCV) genotypes and subtypes were determined in hemodialysis patients in the Federal District, Brazil, by sequencing of the 5' noncoding (NC) and nonstructural 5B (NS5B) regions. From 761 patients, 66 anti-HCV-positive samples were tested for HCV RNA. All 51 HCV RNA-positive samples by PCR of the 5' NC region were genotyped as genotypes 1 (90.2%) and 3 (9.8%). Subtype 1a (82.3%) was the most prevalent, followed by subtypes 3a (9.8%), 1b (5.9%) and 1a/1b (2.0%). Forty-two samples could be amplified and genotyped in the NS5B region: 38 (90.5%) as genotype 1, subtypes 1a, and 8 (9.5%) as genotype 3, subtype 3a. For the 42 samples sequenced in both regions, the genotypes and subtypes determined were concordant in 100% and 95.2% of cases, respectively. Two samples presented discrepant results, with the 5' NC region not distinguishing correctly the subtypes 1a and 1b. These findings indicate that the HCV genotype 1, subtype 1a, is the most prevalent among hemodialysis patients in the Federal District, Brazil.

KEYWORDS: Hepatitis C virus; Hemodialysis; Genotypes.

INTRODUCTION

Hemodialysis patients are at high risk of acquiring the hepatitis C virus (HCV) infection. These patients have an increased tendency to become HCV chronic carriers and also to be a potential reservoir for its transmission, possibly contributing to the nosocomial spread of HCV in dialysis centers \(^5,13\). In addition, hepatitis C seems to increase the mortality rate in this group of patients \(^4,20\).

Based on the nucleotide (nt) sequence divergence, HCV is classified into six major genetic groups, designated genotypes (1 to 6), each one comprising multiple subtypes (designated a, b, c, etc). These genotypes and subtypes have distinct geographical distributions. Genotype determination is a relevant predictive parameter of the response to the antiviral treatment. Information about subtype distribution is required to perform effective HCV molecular and epidemiological surveillance, to study modes of transmission, and improve further vaccine development efforts \(^6,12\).

In order to determine HCV genotypes and subtypes, the choice of the genome region to be analyzed is crucial. This region must present genotype-specific and subtype-specific motifs. Additionally it must be highly conserved to be detected by most of the available assays, based on nucleic acid amplification. Several assays were developed to identify HCV genotypes and subtypes from the 5’ non-coding (NC) region because this region is readily amplified by PCR. However, this region does not contain sufficient information to resolve subtypes. Therefore, sequence analysis of the protein-coding regions such as Core, Envelope (E1) or nonstructural 5B (NS5B) of HCV genome are necessary to discriminating between subtypes \(^8,14,22\).

In Brazil, a country of continental dimensions, the distribution of HCV genotypes and subtypes among hemodialysis patients has not been well documented. Studies carried out in São Paulo \(^15,16,19\), Rio de Janeiro \(^1\), Belo Horizonte \(^7\), Recife \(^1\), Tocantins \(^23\), Goiás \(^8\) and Campo Grande \(^10\) have shown that genotype 1, subtype 1a, was prevalent in those patients. However, the genotyping methods employed in the majority of those studies (except in Goiás State), which were based on 5’ NC region analyses, did not permit the correct identification of the HCV subtypes. In the Federal District, Brazil, there are no data about the genetic diversity of HCV isolated in these patients. In the present study, the HCV genotypes and subtypes were determined in hemodialysis patients in the Federal District, Brazil, by nucleotide sequencing analysis of the 5’ NC and NS5B regions.

MATERIALS AND METHODS

The study was carried out in the seven dialysis units of the Federal

RESULTS AND DISCUSSION

The HCV RNA was detected in 51 samples by RT-nested PCR of the 5'NC region. Direct nucleotide sequencing of 207 nt from the 5'NC region was performed in all HCV RNA-positive samples. Among them, 90.2% were of genotype 1, subtypes 1a (82.3%), 1b (5.9%) and 1a/1b (2.0%). The remaining samples (9.8%) belonged to genotype 3, subtype 3a (Table 1).

Of the 51 HCV RNA-positive samples, 42 (82.3%) samples could be amplified and sequenced in the NS5B region. Using phylogenetic tree analysis of this region 328 nt, 38 sequences (90.5%) were classified as genotype 1, subtype 1a. All genotype 3 sequences (n = 4; 9.5%) were grouped inside the clad of subtype 3a of the phylogenetic tree (Fig. 1 and Table 1).

For the 42 samples which were genotyped by sequencing of the two regions, the genotypes and subtypes determined were concordant in 100% and 95.2% of cases, respectively. Two samples showed discrepant results. These samples (subtype 1b by the analysis of the 5' NC region) were identified as 1a by sequence analysis of the NS5B region. In addition, the 9 samples which were amplified and sequenced in the 5'NC region, were of subtype 1a (n = 6), 1b (n = 1), 1a/1b (n = 1) and 3a (n = 1).

The distribution of HCV genotypes in the study population, with a predominance of subtype 1a (90.5%) followed by 3a (9.5%), differs from that of local blood donors, in which subtypes 3a (39.1%), 1a (34.1%) and 1b (26.8%) were detected. However, the predominance of genotype 1, subtype 1a was also found in other Brazilian studies and sequenced in the 5' NC region, but not in the NS5B region, were of subtype 1a (n = 6), 1b (n = 1), 1a/1b (n = 1) and 3a (n = 1).

Table 1

<table>
<thead>
<tr>
<th>Region/Subtype</th>
<th>N</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5' NC (N = 51)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1a</td>
<td>42</td>
<td>82.3</td>
</tr>
<tr>
<td>1b</td>
<td>3</td>
<td>5.9</td>
</tr>
<tr>
<td>1a/1b</td>
<td>1</td>
<td>2.0</td>
</tr>
<tr>
<td>3a</td>
<td>5</td>
<td>9.8</td>
</tr>
<tr>
<td>NS5B (N = 42)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1a</td>
<td>38</td>
<td>90.5</td>
</tr>
<tr>
<td>1b</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3a</td>
<td>4</td>
<td>9.5</td>
</tr>
</tbody>
</table>

In conclusion, by means of 5'NC and NS5B sequence analysis, this study demonstrates that HCV subtype 1a is the most prevalent among hemodialysis patients in the Federal District, Brazil. In addition, the results presented here highlight that 5' NC and NS5B regions provide...
Os genótipos e subtipos do vírus da hepatite C (HCV) foram determinados em pacientes em hemodiálise no Distrito Federal, Brasil, pelo sequenciamento das regiões 5’-NC não codificante (NC) e não estrutural 5B (NS5B). De 761 pacientes, 66 amostras anti-HCV positivas foram testadas para RNA-HCV. Todas as 51 amostras RNA-HCV positivas por PCR para a região 5’-NC foram genotipadas como dos genótipos 1 (90,2%) e 3 (9,8%). O subtipo 1a (82,3%) foi o mais prevalente, seguido pelos subtipos 3a (9,8%), 1b (5,9%) e 1a/1b (2,0%). Quarenta e duas amostras puderam ser amplificadas e genotipadas na região NS5B: 38 (90,5%) como genótipo 1, subtipo 1a, e 8 (9,5%) como genótipo 3, subtipo 3a. Para as 42 amostras sequenciadas nas duas regiões, os genótipos e subtipos determinados foram concordantes em 100% e 95,2% dos casos, respectivamente. Duas amostras apresentaram resultados discrepantes, sendo que a região 5’-NC não diferenciou corretamente os subtipos 1a e 1b. Estes achados indicam que o genótipo 1, subtipo 1a, do HCV é o mais prevalente em pacientes em hemodiálise no Distrito Federal, Brasil.

**RESUMO**

Genótipos do vírus da hepatite C em pacientes em hemodiálise no Distrito Federal, Brasil

Os genótipos e subtipos do vírus da hepatite C (HCV) foram determinados em pacientes em hemodiálise no Distrito Federal, Brasil, pelo sequenciamento das regiões 5’-NC não codificante (NC) e não estrutural 5B (NS5B). De 761 pacientes, 66 amostras anti-HCV positivas foram testadas para RNA-HCV. Todas as 51 amostras RNA-HCV positivas por PCR para a região 5’-NC foram genotipadas como dos genótipos 1 (90,2%) e 3 (9,8%). O subtipo 1a (82,3%) foi o mais prevalente, seguido pelos subtipos 3a (9,8%), 1b (5,9%) e 1a/1b (2,0%). Quarenta e duas amostras puderam ser amplificadas e genotipadas na região NS5B: 38 (90,5%) como genótipo 1, subtipo 1a, e 8 (9,5%) como genótipo 3, subtipo 3a. Para as 42 amostras sequenciadas nas duas regiões, os genótipos e subtipos determinados foram concordantes em 100% e 95,2% dos casos, respectivamente. Duas amostras apresentaram resultados discrepantes, sendo que a região 5’-NC não diferenciou corretamente os subtipos 1a e 1b. Estes achados indicam que o genótipo 1, subtipo 1a, do HCV é o mais prevalente em pacientes em hemodiálise no Distrito Federal, Brasil.

**REFERENCES**

1. Albuquerque ACC, Coelho MBCD, Lopes EPA, Lemos MF, Moreira RC. Prevalence and risk factors of hepatitis C virus infection in hemodialysis patients from one center in Recife, Brazil. Mem Inst Oswaldo Cruz. 2005;100:467-70.


