Distribution of hepatitis C virus genotypes among different exposure categories in the State of Pará, Brazilian Amazon

Distribuição dos genótipos do vírus da hepatite C em diferentes categorias de exposição no Estado de Pará, Amazônia Brasileira

Leila Sawada1,2, Andréia Cristina Costa Pinheiro1,2, Daiane Locks1,2, Adriana do Socorro Coelho Pimenta3, Priscila Rocha Rezende2, Deborah Maia Crespo3, José Ângelo Barletta Crescente4, José Alexandre Rodrigues de Lemos3,4 and Aldemir Branco de Oliveira Filho1,2,5

ABSTRACT

Introduction: Epidemiological studies concerning HCV genotypic distribution in the Brazilian Amazon are scarce. Thus, this study determined the patterns of distribution of HCV genotypes among different exposure categories in the State of Pará, Brazilian Amazon. Methods: A cross-sectional study was conducted on 312 HCV-infected individuals belonging to different categories of exposure, who were attended at the HEMOPA, CENPREN and a private hemodialysis clinic in Belém. They were tested for HCV antibodies using an immunoenzymatic test, RNA-HCV, using real-time PCR and HCV genotyping through phylogenetic analysis of the 5' UTR. The population groups were epidemiologically characterized according to data collected in a brief interview or medical consultation. Results: Genotype 1 predominated in all the different categories of HCV exposure. HCV genotypic distribution among blood donors comprised genotypes 1 (94%) and 3 (6%). All patients with chronic hematologic diseases had HCV genotype 1. The genotypic distribution in illicit-drug users comprised genotypes 1 (59.6%) and 3 (40.4%). In patients under hemodialysis, genotypes 1 (90.1%), 2 (3.3%), and 3 (6.6%) were detected. Finally, the frequency of genotypes 1 and 3 was significantly different between the groups: BD and DU, PUH and DU, PUH and PCHD and PCHD and DU. Conclusions: The genotypic frequency and distribution of HCV in different categories of exposure in the State of Pará showed a predominance of genotype 1, regardless of the possible risk of infection.

Keywords: HCV. Genotype. Risk group. Pará. Brazilian Amazon.

INTRODUCTION

Hepatitis C virus (HCV) infection is a global health problem. HCV is one of the leading causes of acute and chronic hepatitis, cirrhosis of the liver and hepatocellular carcinoma1. The prevalence of HCV infection varies from 0.1% to 0.2% in the West, to 3% in Mediterranean countries and well over 10% in some countries and regions of Africa, Asia and Europe, such as the Chinese provinces of Hubei (30.1%) and Mongolia (31.9%), and in Egypt (28%)1,2. Official data on the prevalence of HCV in the Brazilian population are limited. It is estimated that the seroprevalence in blood donors is approximately 1.6%, characterizing Brazil as an area with low endemicity. The HCV prevalence in northern Brazil (Brazilian Amazon) is the highest among the Brazilian regions (2.2%) and in this region the highest rates are observed in the States of Acre (5.9%) and Pará (0.2 - 2%)3,5. Phyllogenetic analysis of nucleotide sequences resulted in the classification of six genotypes and several subtypes. HCV genotypes and their subtypes coexist in various geographical locations, but show different prevalence levels6-7. The prevalence of genotypes is also associated with the transmission route of the infection. For example, infections with subtypes 1a and 3a have been shown to be significantly associated with intravenous drug abuse8. In addition, HCV genotypes possess different biological potentials. Certain HCV genotypes are more amenable to interferon treatment and are more frequently associated with severe forms of liver disease9,10. In Brazil, genotype 1 predominates, followed by genotypes 3 and 21,11,12. However, epidemiological studies concerning HCV genotypic distribution in the Brazilian Amazon are rare. The HCV genotype most commonly identified throughout most of the states of Acre (health-care workers and chronic hepatitis C patients), Amazonas (chronic hepatitis C patients), and Pará (blood
donors) is genotype 1, at 78%, 64% and 93%, respectively\textsuperscript{5,12,13}. This work reports on the patterns of distribution of HCV genotypes among patients in different categories of exposure in the State of Pará, Brazilian Amazon.

**METHODS**

Individuals with HCV infection who were attended by the Blood Bank of Pará (HEMOPA\textsuperscript{5}), the Center for Prevention and Treatment of Chemical Dependency (CENPREN) and a private hemodialysis clinic, from January 2004 to February 2008 in the State of Pará, Brazilian Amazon, were selected. The population groups were epidemiologically characterized according to data collected in a brief interview or medical consultation. All of the plasma samples were tested for HCV antibodies using an immunoenzyme test (Murex anti-HCV version 4.0, Murex Biotech SA, Kyalami, South Africa). A confirmatory test for viral infection was performed on the seroreactive samples using real-time PCR (ABI Prism 7,000, Applied Biosystems). Viral RNA was extracted using the commercial extraction kit QIAamp Viral RNA Mini Kit (Qiagen). Molecular diagnosis was based on detection of the 67 base pairs of 5' UTR with the commercial kit TaqMan EZ RT-PCR Core Reagents (Applied Biosystems)\textsuperscript{5}. Following molecular diagnosis of viral infection, all positive samples were selected for amplification of the 5' UTR using nested-PCR\textsuperscript{12}. The product of this second amplification was run on a 2% agarose gel buffered with TBE; the gel was stained with ethidium bromide and observed under ultraviolet light. The amplified fragment was sequenced in both directions using the dideoxynucleotide chain terminator method with an ABI Prism 377 and the commercial kit Big Dye Cycle Sequencing Standard, both from Applied Biosystems.

All nucleotide sequences obtained were edited and aligned using BioEdit software (http://www.mbio.ncsu.edu/BioEdit/bioedit.htm). This alignment was entered in the DnaSP software version 5.10\textsuperscript{14} to identify likely identical nucleotide sequences, and in the Modelgenerator software version 0.85\textsuperscript{15} to select the best model to apply to phylogenetic analyses, according to the corrected Akaike information criterion. These parameters were used in the PHYML program version 2.4.4\textsuperscript{16} to infer trees, according to the maximum-likelihood method. To test the robustness of the tree topologies, 1,000 bootstrap replicates were performed. The final phylogenetic tree was obtained by majority-rule consensus and then edited using the graphical resources contained in the FigTree software version 1.3.1 (http://tree.bio.ed.ac.uk/software/figtree). Nucleotide sequences obtained from the National Center of Biotechnology Information were added to the alignment and used to construct the phylogenetic tree to identify HCV genotypes (Genotype 1: U45476, AJ132997, M62321. Genotype 2: D49757, D49754, AB030907, D10077, D49745, AB031663, D00944, AF169003, D50409, D49755, AY746460. Genotype 3: D17763, D28917, D37840, D49374, D49774, D49753. Genotype 4: D45193. Genotype 5: D50466. Genotype 6: D88476, D88473, D88475). Univariate analyses, comparing the distribution of HCV genotypes among the different exposure categories, were performed using BioEstat software version 5.0. The Chi square test was used and the results were considered to be statistically significant when $p < 0.05$. Nucleotide sequence data obtained in this work are available in the DDBJ/EMBL/GenBank databases under the accession numbers FJ696418 to FJ696533, and HM042983 to HM043178.

**RESULTS**

The different HCV exposure categories consisted of 151 blood donors who were selected from 298,259 donors attended at the HEMOPA, 47 of the 94 illicit drug users under the care of the CENPREN, 53 of the 98 patients with chronic hematological disease who were receiving treatment at the HEMOPA and 61 of the 271 patients under hemodialysis in a private hemodialysis clinic, located in the City of Belém. A total of 312 nucleotide sequences of HCV 5' UTR were isolated; however, 293 identical nucleotide sequences were identified and only one representative was maintained in the alignment. Thus, the final alignment consisted of 19 nucleotide sequences: 5 isolates in blood donors, 5 isolates in illicit drug users, 3 isolates in patients with chronic hematological disease and 6 isolates in patients under hemodialysis.

Based on the Modelgenerator software, the most appropriate evolutionary model for this data matrix was the Tamura-Nei model, adjusted by the parameters proportion of invariant sites (0.451) and rate of gamma distribution (0.327). The base frequencies $A = 0.22459, C = 0.27331, G = 0.27534, T = 0.22677$, transition/transversion ratio for purines (3.923) and transition/transversion ratio for pyrimidines (4.885) were estimated by the PHYML software during the phylogenetic analysis. Of the different HCV exposure categories, genotype 1 predominated (Figure 1 and Table 1). A likely predominance of subtype 1b was also detected in all the risk groups. Due to the low values of grouping, it is necessary to analyze another region of the virus genome to confirm this finding.

The group of infected donor blood was comprised mostly of males (66%), with a significantly higher frequency of infection in individuals aged 30 to 49 years-old (30-39 years-old: 27%; 40-49 years-old: 39%). The genotypic distribution was formed by genotypes 1 (approximately 94%) and 3 (approximately 6%) (Figure 1 and Table 1). The group of infected multitransfused patients had one of the following chronic hematological diseases: hemophilia A (52%), hemophilia B (12.3%), sickle-cell anemia (20.4%), deficiency of coagulation factors (8.2%) and others (7.1%). The average age of infected patients was 36 years-old (23-56 years-old), and the average number of blood transfusions or blood products received was around 45 (min-max: 6-352). All these patients presented HCV genotype 1 (Figure 1 and Table 1). The group of infected drug users consumed preferably non-injected drugs (82.1%) including marijuana, crack cocaine, and cocaine. Most drug users were males (62.3%), with an average age of around 28 years-old (18-52 years-old). Among the drug users, the genotypic distribution was formed by genotypes 1 (59.6%) and 3 (40.4%) (Figure 1 and Table 1). In patients under hemodialysis, genotypes 1 (90.2%), 2 (3.3%), and 3 (6.5%) were detected. Most patients under hemodialysis were males (66.6%), with an average age around 47 years-old (28-65 years-old). The mean period that they had received dialysis therapy was 1.5 years (min-max: 0.25-5.5 years) (Figure 1 and Table 1). Finally, the HCV genotypic frequency (1 and 3) was significantly different between the groups: blood donors and illicit drug users ($\chi^2 = 18.58, p$-value $< 0.001$), hemodialysis patients and illicit drug users ($\chi^2 = 13.83, p$-value $< 0.001$), hemodialysis patients and multitransfused patients ($\chi^2 = 4.87, p$-value $= 0.02$), and multitransfused patients and illicit drug users ($\chi^2 = 25.57, p$-value $= 0.001$). This probably reflects the different routes of viral transmission.
TABLE 1 - Distribution of HCV genotypes among groups of patients in different exposure categories in the Brazilian Amazon.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Genotype 1</th>
<th>Genotype 2</th>
<th>Genotype 3</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>Blood donors</td>
<td>142</td>
<td>94.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Patients with chronic hematological disease</td>
<td>53</td>
<td>100.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Illicit drug users</td>
<td>28</td>
<td>59.6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Patients under hemodialysis</td>
<td>55</td>
<td>90.2</td>
<td>2</td>
<td>3.3</td>
</tr>
</tbody>
</table>

HCV: hepatitis C virus.

FIGURE 1 - Maximum-likelihood tree constructed based on the alignment of 5′ UTR of HCV. The robustness of the tree topologies was evaluated by 1,000 bootstrap replicates (bootstrap values < 50 were not included). The numbers indicate the grouping of the various HCV genotypes (1-6). Identification of different exposure categories to HCV infection in figure. DU: illicit drug users, BD: blood donors, PUH: patients under hemodialysis, PCHD: patients with chronic hematological disease. Genotype 1: U45476, AJ132997, M62321. Genotype 2: D49757, D49754, AB030907, D10077, D09444, AB031663, D49757, D49754, PUH55, PUH56, D49745, D50409, AF169003, D49755, AY746460. Genotype 3: D17763, D28917, D37840, D49374, D14166, PUH59. Genotype 4: D45193. Genotype 5: D50466. Genotype 6: D88476, D88473, D88475.
DISCUSSION

Some genotypes of HCV (1a, 1b, 2a, 2b, and 3a) are widely distributed all over the world. Others have a more restricted distribution, such as genotype 4 in the Middle East and Africa, genotype 5a in South Africa and genotype 6 in Southeast Asia. Certain factors, such as migration and travel patterns, may alter the current genotypic map. In Sardinia, Coppola et al. identified genotype 4, which was rare in continental Italy, at a level of 1%, and suggested that this high prevalence was associated with a connection between Northern Africa and the Southern Mediterranean basin with the nearby Italian island. In Brazil, genotype 1 predominates, followed by genotypes 3 and 2. The distribution of HCV genotypes in different exposure categories in Brazil is similar, with a higher frequency of genotypes 1 and 3, followed by genotype 2 and sporadic cases of genotype 4. This study showed a predominance of genotype 1, followed by genotypes 3 and 2, while genotypes 4 and 5 were not detected. This result probably reflects the high frequency of genotype 1 and 3 in the Brazilian Amazon. Furthermore, the risk of HCV infection between the groups was observed in the distribution and frequency of the viral genotypes. The high frequency of genotype 1 in blood donors influenced the complete dominance of genotype 1 in multitransfused patients, which was probably transferred by blood/blood product transfusion. In the Netherlands, the transfusion of blood/blood products was responsible for infection in 17.5% of genotype 1 patients. Among patients with genotype 1b, transfusion of blood/blood products was the main route of transmission.

Some studies have reported that changes may occur in the HCV subtype pattern. In some countries of Europe, genotype 1a and 3a are increasing, while 2a, 2c and 1b are decreasing, especially in young patients. These results probably stem from the high prevalence of genotype 1a and 3a among intravenous drug users, the reservoir from which the general population is then affected. Other studies have reported that a growing number of young drug addicts are infected with subtype 3a. These results indicate that changes in social behavior affect the epidemiological trends of infectious diseases to a significant extent. In the present study, these epidemiological characteristics can be seen in the group of users of illicit drugs, reflected in the high frequency of genotype 3 (3a/3b) and the low average age of those infected. In the group of patients under hemodialysis, the longer exposure time (the higher average age) to possible risk factors may have been responsible for this group showing the highest genotypic diversity of HCV.

This study also provided evidence that the 5' UTR is conserved and limited in its ability to discriminate subtypes within genotypes 1, 2, 3, 4, and 6. The 5' UTR sequence has been described. Despite this, the 5' UTR contains sufficient variation to resolve HCV classifications at the level of viral genotype. Thus, this study illustrated the need for sequencing of other regions of the HCV genome to improve the resolution of viral subtypes.

Based on the results of this study, it can be concluded that most hepatitis C infections in the Brazilian Amazon consist of genotype 1, regardless of the possible risk factors for infection. This implies that patients infected with HCV in the Brazilian Amazon should preferentially be treated with a specific clinical protocol for genotype 1. Moreover, this study shows the need for sequencing of other regions of the HCV genome, to achieve improved resolution of the viral subtypes circulating in the Brazilian Amazon. In the future, more extensive surveys should be conducted to assess geographical differences in the distribution of HCV genotypes among different exposure categories in other Brazilian regions.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

FINANCIAL SUPPORT

FAPESP, PPSUS, CNPq, and PN-DST/AIDS.

REFERENCES

14. Sawada L et al. - HCV genotypes among different exposure categories, Pará, Brazil.