RENAL FAILURE

CLINICAL STUDY

Molecular Investigation of GB Virus C RNA in Hemodialysis and Thalassemics Patients from Brazil

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ABSTRACT

The GB virus C (GBV-C)/hepatitis G virus (HGV) is a member of the Flaviviridae family. Based on the clinical and epidemiological profiles, this virus could be acquired mainly by parenteral transmission through contaminated blood. We therefore investigated the presence of GBV-C/HGV and its relation with the other blood borne viruses as hepatitis B and C viruses (HBV, HCV) in hemodialysis and thalassemic individuals and blood donors from Ribeirão Preto—Brazil. Detection of blood borne virus markers including HBV surface antigen (HbsAg),

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HBV core antibody (anti-Hbc) and HCV antibody was carried out. HIV-1, HIV-2, HTLV-1 and HTLV-2 were also investigated. GBV-C/HGV RNA was detected by reverse transcriptase and polymerase chain reaction (RT-PCR). Ninety-four serum samples from patients with chronic renal failure were analyzed. GBV-C/HGV RNA was identified in 12 (12.8%) patients, anti-HCV antibodies in 28 (29.8%), anti-Hbc in 9 (9.6%), anti-HIV in 1 (1%), HBsAg in 33 (35.1%), and HBsAg/anti-Hbc was observed in 2 (2.1%) patients. Thirty-six (38.3%) samples were non-reactive. Seven of the 12 GBV-C/HGV RNA infected samples were co-infected with other viruses: 3 (25%) with HBsAg, 2 (16.7%) with anti-HCV and 2 (16.7%) with anti-Hbc/anti-HCV/HBsAg. Among the 42 thalassemic patients, GBV-C/HGV RNA was detected in 6/42 patients (14.2%). Three patients presented GBV-C/HGV, with other blood borne markers. We also detected GBV-C/HGV in 6/50 (12%) blood donors. In these GBV-C/HGV positive thalassemics patients, 50% (3/6) were young individuals (lesser 15 years old) and 67% (4/6) were female patients. The presence of GBV-C RNA in the absence of hepatitis B and C infection in the young patients and healthy donors could be indicate that this virus is capable of independent transmission and does not contribute to liver disease.

Key Words: HGV; HBV; HCV; Blood-borne diseases; Chronic renal failure; Thalassemia; GBV-C/HGV; HCV; HBV; Blood donors.

INTRODUCTION

GB virus C/hepatitis G virus (GBV-C/HGV) is believed to be a member of the Flaviviridae, which includes hepatitis C virus (HCV).[1,2] The flavivirus GBV-C, also designated hepatitis G virus (HGV) was identified in a search for hepatitis viruses, but no disease is currently known to be associated with it. GBV-C and HGV are closely related isolates of the same virus, with more than 95 percent sequence homology. We use the name GBV-C, since it currently appears that the virus is not a cause of hepatitis. Persistent infection by GBV-C is common in humans, and genetically divergent isolates have been identified in different parts of the world.[3] GBV-C and HCV are thus related and share a common ancestor according to phylogenetic analysis.[4] Although GBV-C and HCV both belong to the Flaviviridae family, the overall genomic structure of GBV-C is not similar to that of HCV.[5] They have only about 29% amino acid identity and therefore GBV-C is clearly distinct from HCV.[4] Some authors detected co-infection with GBV-C and HCV was 58% and with GBV-C and HBsAg was 41.6%, which could indicate a common route of transmission of two viruses, reported previously.[5] In the past years HCV, would be cause chronic hepatitis, cirrhosis and possibly hepatocellular carcinoma[6] but the clinical significance of GBV-C infection and its pathogenic role in hepatitis or any other disease remain unclear.[7] It is possible that GBV-C may be linked to extrahepatic disease.[6]

It is known that GBV-C clusters in particular populations typified by hemodialysis patients, thalassemic, hemophiliacs and intravenous drug users,[9–13] On the other hand, many investigators showed that this infection does not contribute to liver disease.[7] There are little information about the epidemiology, mode of
transmission and pathology associated with GBV-C and conflicting reports regarding association with disease have published. In this study, we investigated the prevalence of GBV-C in relation of the other blood borne viruses as HBV, HCV, HIV-1, HIV-2, HTLV-1 and HTLV-2 in Brazilian thalassemic and hemodialysis patients and blood donors.

METHODS

Following approval from the Human Ethics Committee of the University of São Paulo, blood samples were obtained from patients and blood donors.

Plasma Samples

Hemodialysis Patients

We collected 94 plasma samples from patients with chronic renal failure undergoing dialysis treatment and 42 thalassemic patients. Hepatitis B surface antigen markers (HBsAg), virus B anti-core antibody (Anti-Hbc), anti-hepatitis C antibody (anti-HCV) and anti-human immunodeficiency virus antibody (anti-HIV 1/2) were determined. All analyses were performed at the Ribeirão Preto Blood Center, University of São Paulo, Brazil.

Mean age of the patients with chronic renal failure was 43.5 (± 13.6) years, 43 patients were females and 51 males. The patients presented a mean duration of dialysis of 46.3 (± 40.4) months and a mean transfusion number of 4.3 (± 4.0).

Blood donors: Plasma samples from healthy donors were also collected from health individuals.

Serological Tests

Sorology Department from Regional Hemocentro of Ribeirão Preto, São Paulo—Brazil, performed serological assay. Serum samples were examined for HBsAg and Hbc by Hepanostika HBsAg and Hepanostika anti-Hbc, Uni-Form-MicroElisa System—Organon-Teknika. HCV was determined by Ortho HCV-3.0 ELISA Test System with enhanced save—Ortho. HIV-1/HIV-2 and HTLV-1/HTLV-2 were determined by Ab-Capture ELISA test system—Ortho and Uni-Form 2 plus O—Vironostika—Organon-Teknika.

RNA Extraction

GBV-C/HGV RNA was extracted from 250μL of plasma sample using TRIzol-LS Reagent (GIBCO-BRL, Grand Island, NY, USA) according to the manufacturer’s instructions.
Reverse transcription was carried out with 50 pmol of the type-specific outer antisense primer using 50 units of cloned murine Leukemia Virus (MuLV) reverse transcriptase (Perkin Elmer) GeneAmp RNA PCR kit (Part number N808-0017) at 42°C for 60 min (50 mM Tris–HCl pH 8.3, 75 mM KCl, 3 mM MgCl₂, 200 µM of dNTP).

PCR Conditions

The HGV primers were used for the amplification of HGV RNA. The HGV primers were designed based on the sequence of HGV (GenBank sequences U44402) and were targeted to amplify the conserved 5’ end (5’UTR) of the HGV genome (outer sense 5’GGTAGGTCGTAAATCCCGGT 3’; outer antisense 5’CCCACTGGTCCTTGTCAACT 3’; inner sense 5’TGGTAGCCACTATAGGTGG 3’; inner antisense 5’GCCTATTTGTTCAAGAG 3’). Reaction conditions for the two PCR rounds were the same (20 mM Tris–HCl pH 8.4, 50 mM KCl, 1.5 mM MgCl₂, 200 µM dNTP and 1.25 units of Taq polymerase) and consisted of an initial denaturation step of 94°C for 5 min followed by 40 cycles of 94°C for 1 min, 45°C for 1 min and 72°C for 1 min and a final extension of 72°C for 10 min. PCR products of 217 base pairs were analyzed by electrophoresis on a 2% agarose gel and visualized by UV fluorescence after staining with ethidium bromide. The specificity of the detection was analyzed by direct sequencing (373 DNA sequencer, ABI PRISM™—Perkin Elmer) of the PCR products.

Statistical Analysis

The Student t-test was applied to samples obtained from patients with anti-Hbc and anti-HCV antibodies against HGV to test for statistical significance between duration of dialysis and number of transfusions.

RESULTS

Ninety-four samples from the patients undergoing hemodialysis were analyzed. Among reactive samples, 33 (35.1%) of them for HBsAg and 9 (9.6%) for anti-Hbc; samples from two patients were positive for both markers, suggesting chronic hepatitis B virus infection. Twenty-eight (29.8%) samples were positive for anti-HCV and 1 (1%) for HIV. GBV-C RNA was detected in 12 samples (12.8%). Thirty-six (38.3%) samples were negative for all markers (Table 1).

GBV-C co-infection with hepatitis B virus was detected in three cases (25%), and HGV/HCV in 2 (16.7%), with the samples of 2 (16.7%) patients being concomitantly reactive for HGV, HBsAg and HCV. Five (41.6%) samples were positive
Table 1. Hepatitis B, C, G and HIV 1/2 markers detected in 94 samples obtained from hemodialysis patients.

<table>
<thead>
<tr>
<th>Samples</th>
<th>HBsAg</th>
<th>Anti-HBc</th>
<th>HbsAg/Anti-HBc</th>
<th>Anti-HCV</th>
<th>Anti-HIV</th>
<th>GBV-C RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>94</td>
<td>33 (35.1%)</td>
<td>9 (9.6%)</td>
<td>2 (2.1%)</td>
<td>28 (29.8%)</td>
<td>1 (1%)</td>
<td>12 (12.8%)</td>
</tr>
</tbody>
</table>

Table 2. Frequency of isolated GBV-C RNA associated infections with other hepatitis-causing viruses.

<table>
<thead>
<tr>
<th>HBsAg</th>
<th>Anti-HCV</th>
<th>Anti-HBc/HBsAg/Anti-HCV</th>
<th>Isolated GBV-C RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 (25%)</td>
<td>2 (16.7%)</td>
<td>2 (16.7%)</td>
<td>5 (41.6%)</td>
</tr>
</tbody>
</table>

Table 3. Prevalence of GBV-C RNA and hepatitis B and C markers in hemodialysis patients in relation to mean dialysis duration, number of transfusions and age.

<table>
<thead>
<tr>
<th>Viral marker</th>
<th>Number of patients</th>
<th>Duration of hemodialysisa (months)</th>
<th>Number of transfusionsa</th>
<th>Agea</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBsAg</td>
<td>33</td>
<td>50.7 (±42.6)</td>
<td>4.3 (±3.8)</td>
<td>44.8 (±15.1)</td>
</tr>
<tr>
<td>Anti-HBc</td>
<td>9</td>
<td>62.1a (±41.2)</td>
<td>6.6a (±4.3)</td>
<td>31.5 (±8.7)</td>
</tr>
<tr>
<td>Anti-HCV</td>
<td>28</td>
<td>69.3a (±48.6)</td>
<td>5.8a (±4.1)</td>
<td>43.3 (±12.4)</td>
</tr>
<tr>
<td>HGV</td>
<td>12</td>
<td>46.5 (±42.6)</td>
<td>4.75 (±3.7)</td>
<td>45.9 (±14.1)</td>
</tr>
</tbody>
</table>

Data are reported as mean and standard deviation.

*p > 0.05, not significantly different from GBV-C RNA (Student t-test).

for HGV only (Table 2). The GBV-C products from the three positive patients were directly sequenced and were shown to be compatible to that of GBV-C (U44402).

Table 3 shows the prevalence of hepatitis B, C and G markers used in the present study in relation to dialysis duration and the number of transfusions. The prevalence of HGV, HCV and HBV increased after 40 months of hemodialysis (data not shown). Mean hemodialysis duration as well as mean transfusion number did not differ significantly between HGV-positive patients and individuals positive for anti-Hbc and anti-HCV (p > 0.05, Student t-test).

We also analyzed 42 samples from thalassemics patients. All patients were negative to HBs Ag, HIV-1, HIV-2, HTLV-1 and HTLV-2. The age from our thalassemic Brazilian patients in the majority was children and young people. GBV-C was detected in 6 (14.2%) patients as demonstrated in the Table 4.

**DISCUSSION**

High prevalence of GBV-C is observed in both rural and urban Brazilian populations from which blood donors and organs are obtained.[14–16]
Prevalence of GBV-C (15%) was observed in 66 chronic renal failure patients undergoing dialysis,\(^{[17]}\) a value similar to that observed in the present study (12%). However, it is interesting to note that five patients presented isolated GBV-C infection (Table 2), and 10 (83.3%) of the 12 HGV-positive hemodialysis patients were females (data not shown).

No co-infection with HIV was observed, and the most frequent co-infection (25%) was with HBV, which was detected in 35.1% of the samples. No statistically significant correlation with dialysis duration or number of transfusions was observed, demonstrating that GBV-C infection occurs through routes other than the transfusion one. In this context, several authors\(^{[18–21]}\) have reported nosocomial infection.

HBV and HCV are major infective agents in transfusion dependent patients.\(^{[19]}\) We found 14.2% GBV-C RNA positivity in Brazilian thalassemics patients. Among these GBV-C RNA positive individuals we detected one patient with anti-HBc and anti-HCV which could present relation with the time of transfusion, and other 18 years old HGV positive thalassemic patient presented anti-HCV antibodies.

Recently was GBV-C RNA were detected in 35% from 37 thalassemic patients from young adult thalassemic patients.\(^{[22]}\)

We found absence of HCV and HBV in the 4 (66.6%) GBV-C thalassemic individuals presenting 13 years lesser.

We also detected GBV-C in healthy individuals (12%). The reason for the relatively high prevalence of GBV-C in blood donor populations worldwide need yet to be determined.\(^{[23,24]}\) The prevalence of HGV in blood donors in Western countries is reported to be high.\(^{[2,25]}\)

It has been verified that high titered of maternal viremia and mode of delivery are closely associated with the mother-to-infant transmission of GBV-C/HGV to infants, and the infection usually becomes persistent.\(^{[26]}\)

Various reports indicate varying prevalences of GBV-C infection in populations from around the world, ranging from 0.9% in Japan\(^{[27]}\) to 12.9% in a group of commercial blood donors from the USA, with intermediate figures from volunteer donors in Australia.\(^{[24]}\) It has been reported the prevalence of GBV-C in South African populations.\(^{[28]}\)

Large-scale studies found no causal relationship between GBV-C and its role in disease and there is a strong suggestion that there is another unidentified hepatotropic agent causing non A-E hepatitis.\(^{[29]}\) Nevertheless, no evidence was found that GBV-C coinfection has any impact on the severity of underlying disease.\(^{[30]}\)

### Table 4

Frequency of isolated GBV-C infection and associated infections with other hepatitis-causing viruses Anti-HCV, anti-HBc and GBV-C RNA markers detected in 42 samples obtained from thalassemic patients.

<table>
<thead>
<tr>
<th>Anti-HCV + GBV-C</th>
<th>Anti-HCV + anti-HBc + GBV-C</th>
<th>GBV-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>GBVC (n = 6)</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

\(^{72}\) Watanabe et al.
The study from Ibáñez et al.\cite{31} has been suggested that sexual contact may play a relevant role in the spread of this virus. There is little information about epidemiology route of transmission and pathology associated with GBV-C. Conflicting reports regarding association with disease have been published.

There are results that support the absence of any causal relationship between GBV-C and hepatitis.\cite{10} Our finding could be suggests possible GBV-C transmission in patients with independent infection and no correlation with hepatic pathologies. In spite of various reports supporting that GBV-C is prevalent in the high-risk group of multiple transfused patients our dates with normal donors were similar with thalassemic individuals.

REFERENCES


