GB Virus C Infection in Hemodialysis Patients: Molecular Evidence for Nosocomial Transmission

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Studies of the prevalence and clinical relevance of GB virus C (GBV-C) infection in 328 hemodialysis (HD) patients were done, and the possibility of nosocomial GBV-C transmission was explored by molecular epidemiology methods. For GBV-C viremic patients in a given HD unit, nucleotide sequences of the envelope region were analyzed by phylogenetic tree constructions. Of 328 HD patients, active hepatitis B virus, hepatitis C virus (HCV), and GBV-C infection were detected in 13%, 23%, and 17%, respectively. Except for a higher frequency of HCV coinfection, the demographic and clinical characteristics of patients with and without GBV-C infection were comparable. In contrast, patients with isolated HCV infection had significantly higher serum transaminase levels, longer time on HD, and more blood transfusions. Phylogenetic analysis showed several distinct clusters of closely related GBV-C isolates from one HD unit, suggesting the possibility of nosocomial transmission. These results suggest that GBV-C plays a minimal role in causing hepatitis in Taiwanese HD patients and in nosocomial transmission.

Recently, two flavivirus-like RNA viruses designated GB virus C (GBV-C) and hepatitis G virus (HGV) were independently identified from patients with chronic hepatitis [1, 2]. Both are distantly related to hepatitis C virus (HCV) by phylogenetic analysis, and comparison of full-length sequences indicated that GBV-C and HGV are different isolates of the same virus [3]. The virus is transmitted by blood and blood products and has a global distribution [3]. In addition, patients with chronic hepatitis C are frequently coinfected with GBV-C [2, 3], suggesting that both viruses may share similar modes of transmission. Although the clinical implications of GBV-C remain controversial [4], most studies indicate that GBV-C does not cause liver damage unlike classic hepatitis viruses [3, 5, 6].

Persons on maintenance hemodialysis (HD) are at increased risk of infection with hepatitis viruses [7], and a high prevalence of HCV infection in this at-risk population has been documented [8]. Although blood transfusion is a recognized route of HCV infection in HD patients, the possibility of patient-to-patient or nosocomial transmission has become more evident by detailed epidemiologic and virologic analyses [9, 10]. HD patients have an increased frequency of GBV-C infection compared with the general population [11–13]; however, little is known about the modes of GBV-C transmission other than blood transfusion. In the present study, we investigated the prevalence and clinical relevance of GBV-C infection in a group of Taiwanese HD patients from two different HD units. In addition, we used molecular epidemiology methods to explore the possibility of nosocomial transmission of GBV-C in a given HD unit.

Materials and Methods

Patients. A total of 328 patients (160 men, 168 women; mean age, 54.7 ± 13.6 years) with end-stage renal disease, who were undergoing long-term HD in two HD units in Taipei, were enrolled. They had received HD for a mean of 36.2 ± 39.2 months (range, 1–264). All participants were administered questionnaires to assess possible risk factors for GBV-C infection, including blood transfusion, major operation, intravenous drug usage, and sexual behaviors. Hospital records of the enrolled patients were reviewed for medical history, including elevated serum alanine aminotransferase (ALT) levels and blood transfusions. Elevated serum ALT was defined as >16 IU/L. Serum samples were stored at −70°C until use.

Serologic testing. All serum samples were assayed for hepatitis B surface antigen (HBsAg) by RIA (Ausria-II; Abbott Laboratories, Abbott Park, IL), and antibodies against HCV (anti-
HCV) were determined by a second-generation EIA (Abbott Laboratories).

Detection of viral genomes. Serum HCV RNA was assayed by reverse transcription–polymerase chain reaction (RT-PCR) with nested primers from the most conserved 5′ untranslated region (UTR) of the viral genome. Serum GBV-C RNA was detected by RT-PCR with nested primers derived from the highly conserved 5′ UTR [14].

Amplification and sequencing of the putative E2 region of the GBV-C genome. For serum samples positive for GBV-C RNA from a given HD unit, the putative E2 region of the viral genome was amplified and directly sequenced by use of fluorescence-labeled primers (sequencer model 373A; Applied Biosystems, Foster City, CA) as previously described [15]. To avoid false-positive results, instructions to prevent contamination were strictly followed, and results were considered valid only when they were consistent on at least two separate runs.

Phylogenetic analysis. A phylogenetic tree was constructed by a DNA parsimony program (PHYLIP [Phylogeny Inference Package], version 3.5c; J. Felsenstein, University of Washington, Seattle) based on the nucleotide sequences of the amplified putative E2 region of the GBV-C genome.

Statistical analysis. Data were analyzed by χ² test with Yates’s correction or by Fisher’s exact test, if the expected numbers were <5, and by Student’s t test and by analysis of variance where appropriate. P < .05 was considered significant.

Results

Of 328 patients on HD, HBsAg, anti-HCV, serum HCV RNA, and serum GBV-C RNA were detected in 41 (12.5%), 66 (20%), 48 (14.6%), and 57 (17.3%), respectively. Of 74 patients with evidence of HCV infection, 26 were positive for anti-HCV alone, 40 were positive for both anti-HCV and HCV RNA, and 8 were anti-HCV-negative but viremic. Among the 57 patients positive for GBV-C RNA, 31 (54.4%) were coinfected with other hepatitis viruses: 9 (15.8%) with hepatitis B virus (HBV) and 22 (38.6%) with HCV.

The demographic and clinical characteristics with respect to sex, mean age, positivity of HBsAg, history of increased and peak serum ALT levels, mean duration of HD, and history of transfusion and mean amount of blood products between HD patients with and without GBV-C carriage were comparable (data not shown). However, patients with GBV-C viremia had a significantly higher frequency of HCV infection than those without (39% vs. 17%, P = .004).

The demographic and clinical characteristics of HD patients with isolated HBV, HCV, and GBV-C infection were compared. Patients with HCV infection alone had a more frequent history of serum ALT elevation and a significantly higher mean serum ALT level (table 1). In addition, patients with isolated HCV infection had been on HD longer (P < .001) and received a greater mean amount of blood products (P = .003). The clinical features of patients with GBV-C/HBV or GBV-C/HCV coinfection were similar to those with isolated HBV or HCV infection (data not shown).

To further investigate the genetic relatedness of GBV-C isolates in a given HD unit, nucleotide sequences of the putative E2 region of the viral genome were amplified and determined in 28 GBV-C–viremic patients. These sequences were subsequently included in the phylogenetic analysis, and several distinct clusters of closely related isolates were observed in our phylogenetic tree, consistent with the existence of nosocomial transmission of GBV-C (figure 1).

Discussion

With the discovery of viral genome and subsequent development of molecular diagnostic assays, the epidemiology and clinical significance of GBV-C infection have been extensively studied worldwide [2–6]. GBV-C can be transmitted parenterally, and the infection seems not to cause significant hepatic damage like that caused by classic hepatitis viruses A–E [3, 5, 6]. Our previous studies consistently showed GBV-C viremia of 15%–30% in high-risk groups such as intravenous drug users, hemophiliacs, and polytransfused patients in Taiwan and that

Table 1. Demographic and clinical characteristics of hemodialysis patients with active hepatitis B virus (HBV), hepatitis C virus (HCV), and GB virus C (GBV-C) infection and of those without hepatitis viral infection.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>HBV+ HCV− GBV−</th>
<th>HBV− HCV+ GBV−</th>
<th>HBV+ HCV− GBV−</th>
<th>HBV+ HCV− GBV−</th>
<th>HBV− HCV+ GBV−</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>27</td>
<td>47</td>
<td>30</td>
<td>192</td>
<td></td>
</tr>
<tr>
<td>Male/female</td>
<td>17/10</td>
<td>25/22</td>
<td>12/18</td>
<td>90/102</td>
<td></td>
</tr>
<tr>
<td>Age (years, mean ± SD)</td>
<td>52.5 ± 12.8</td>
<td>54.5 ± 10.8</td>
<td>51.1 ± 15.6</td>
<td>55.7 ± 14.2</td>
<td></td>
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<tr>
<td>Serum ALT (IU/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>History of elevation (%)</td>
<td>5 (18.5)</td>
<td>31 (66.0)</td>
<td>7 (23.3)</td>
<td>18 (9.4)</td>
<td></td>
</tr>
<tr>
<td>Peak level (mean ± SD)</td>
<td>12.2 ± 7.1</td>
<td>26.1 ± 21.3</td>
<td>14.0 ± 13.7</td>
<td>10.1 ± 7.1</td>
<td></td>
</tr>
<tr>
<td>Dialysis duration (months, mean ± SD)</td>
<td>29.9 ± 23.3</td>
<td>72.1 ± 61.3</td>
<td>24.6 ± 19.2</td>
<td>25.7 ± 20.3</td>
<td></td>
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<tr>
<td>Blood transfusion</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>No. (%) of patients</td>
<td>21 (77.8)</td>
<td>40 (85.1)</td>
<td>20 (66.7)</td>
<td>147 (76.6)</td>
<td></td>
</tr>
<tr>
<td>U (mean ± SD)</td>
<td>3.0 ± 2.6</td>
<td>12.3 ± 26.0</td>
<td>3.3 ± 3.8</td>
<td>5.2 ± 9.9</td>
<td></td>
</tr>
</tbody>
</table>

NOTE. +, positive; −, negative; ALT, alanine aminotransferase. Normal value of serum ALT is ≤16 IU/L in this study.

a P < .001.
b P < .001.
c P < 0.01.
d P < .003.
Figure 1. Phylogenetic analysis of GBV-C isolates (G1–G28) from 28 hemodialysis patients with GBV-C viremia based on nucleotide sequences of putative envelope region 2 of the GBV-C genome. Phylogenetic tree was constructed by DNA parsimony program (PHYLIP version 3.5c). Tree is not rooted.

its coinfection does not aggravate the course of chronic hepatitis B or C [6].

In persons on maintenance HD, a defined risk group for hepatitis virus infection, the presence of GBV-C RNA differs widely: 3% in Japan, 16% in Belgium, 19% in Italy, 20% in the United States, and 55% in Indonesia [11, 12]. In this study, the 17% rate of positivity for GBV-C RNA in HD patients was higher than we previously found in healthy controls in Taiwan (1%) [6]. This prevalence of active GBV-C infection in the Taiwanese HD patients is similar to that in the United States and Europe but higher than in Japan. The differences in GBV-C RNA prevalence may be due to differences in experimental techniques (including detection methods for GBV-C RNA, especially the use of different primers [14]), duration of HD therapy, prevalence of blood transfusion history, and geographic factors.

Except for coinfection with HCV, the demographic and clinical characteristics of patients sorted by GBV-C status were similar. Our findings that 39% of the GBV-C–infected HD patients were also positive for anti-HCV and/or HCV RNA imply that GBV-C and HCV share common modes of transmission. Since a significant proportion (54%) of our GBV-C RNA-positive patients were coinfected with HBV or HCV, which may interfere with the interpretation of the clinical implication of GBV-C infection, we examined 3 groups of patients with isolated HBV, HCV, and GBV-C infection and compared them with a group of patients without active hepatitis virus infection (table 1). Our findings that patients positive for HBsAg alone had a comparable duration of HD and blood transfusion history is not surprising, because most HBsAg carriers in Taiwan acquired HBV infection in early childhood. In contrast, patients with isolated HCV infection had been on HD significantly longer and had received a greater mean amount of transfused blood compared with the other 3 groups. These patients had a higher frequency of serum ALT elevation and significantly higher serum ALT levels. These data reconfirm that HCV is the major etiology of liver damage in HD patients [8]. As for patients with GBV-C infection alone, the demographic and clinical features were similar to those of the nonviremic group, suggesting that GBV-C plays a small role in causing hepatitis, as previously reported [5, 6]. Infection with GBV-C may just represent inapparent parenteral transmission in the HD setting.

Several lines of evidence of nosocomial transmission of HCV in HD units have been documented [9, 10]. However, whether nosocomial transmission also occurs with GBV-C, an HCV-like virus, remains unclear. In an earlier study, 1 patient without a history of transfusion had GBV-C RNA sequences identical to those of 2 other patients in the same dialysis unit [12]. This suggests that transmission routes other than blood transfusion, such as patient-to-patient transmission, might be responsible for virus spread. To gain further insight on this important and interesting issue, we studied the genomic diversity and phylogenetic relationship of 28 GBV-C isolates in an HD unit. The analysis was based on the more divergent E2 gene of the viral genome, and our phylogenetic tree showed that several isolates clustered together, indicating close genetic relatedness (figure 1). These data strongly suggest the possibility of patient-to-patient transmission or spread via contaminated common sources. However, the exact mechanisms involved in the transmission of GBV-C in these clusters of patients are unclear, and further studies are needed.

Occasional violations of infection control measures account for most non–transfusion-related HCV infections in HD units [9]. Frequent percutaneous procedures and exposure to blood in such patients provide many opportunities for contamination of surfaces and instruments with virus-contaminated blood. In this environment, an occasional mistake that may be difficult
to recognize could be enough to transmit bloodborne infections from 1 patient to another. Thus, strict infection preventive routines with universal precautions, including the consistent wearing of new gloves whenever patients are treated and the use of chemical sterilization procedures after each dialysis session, should be followed in any HD unit.

In summary, our results suggest that the role of GBV-C in causing hepatitis in HD patients is minimal and that GBV-C coinfection does not worsen the clinical course of chronic HBV and HCV infection. We believe that nosocomial transmission of GBV-C has occurred in our HD units and that universal precautions should be strictly followed to prevent transmission of viruses among HD patients. Although GBV-C seems to be nonpathogenic, it may serve as a marker of nosocomial viral transmission.

References