GBV-C/HGV infection in renal dialysis and transplant patients


A. Szabo¹, S. Viazov², U. Heemann¹, A. Kribben¹, T. Philipp¹ and M. Roggendorf²

¹Department of Nephrology and ²Institute of Virology, Essen University Clinic, Essen, Germany

Abstract

Background. Recently a new human virus (GBV-C/HGV) was identified. With the use of the polymerase chain reaction (PCR) the possibility of a high prevalence of the GBV-C/HGV infection in haemodialysis patients was demonstrated. The aim of the present study was to use a combination of the PCR and a new diagnostic test for antibodies to the viral envelope protein E2 to assess the prevalence of the GBV-C/HGV infection in German patients with renal failure.

Methods. RT-PCR and ELISA were used to detect GBV-C/HGV RNA and antiviral antibodies (anti-E2) in the sera of 31 patients on a maintenance haemodialysis (HD), 25 patients on a peritoneal dialysis (CAPD), and 92 renal transplant patients (RT).

Results. A statistical trend was noted for a higher rate of the GBV-C/HGV RNA in the whole group of patients with renal failure (11.5%) than in the control group of organ donors (5.5%). The difference between GBV-C/HGV RNA prevalence in RT patients (15.2%) and in organ donors (5.5%) was found to be significant. Anti-E2, which are considered as an indicator of a past GBV-C/HGV infection, were detected in 12.9% of HD patients, in 20.0% of CAPD patients, in 10.9% of RT patients, in 11.1% of organ donors, and in 10.9% of blood donors. These differences were not significant. Time on haemodialysis was significantly longer in GBV-C/HGV infected patients compared to uninfected patients and all patients with the GBV-C/HGV RNA have a history of blood transfusions.

Conclusions. Patients with renal failure treated with dialysis or subjected to renal transplantation are at increased risk of acquiring the GBV-C/HGV infection. Higher rates of the GBV-C/HGV RNA and a similar prevalence of anti-E2 in patients with renal failure as compared to donors suggest that the rate of GBV-C/HGV infection resolution in immunosuppressed patients with renal failure might be lower than in immunocompetent patients.

Key words: anti-E2, GBV-C, HGV, haemodialysis, liver disease; peritoneal dialysis, renal transplantation

Introduction

Patients with end-stage kidney disease, treated with dialysis are at high risk of acquiring blood-borne infections such as hepatitis B, D and C. Recently a new infectious agent of humans, GBV-C or hepatitis G virus (GBV-C/HGV), has been identified [1,2]. The GBV-C/HGV genome is a positive-strand RNA of about 9400 kilobases which contains a large open reading frame that encodes a polyprotein precursor of about 2900 amino acids. GBV-C/HGV is a member of the Flaviviridae family and is distantly related to hepatitis C virus (HCV).

There is an increasing evidence that GBV-C/HGV is characterized by similar modes of transmission as HCV [1,3–6]. High prevalence of GBV-C/HGV RNA was detected in several population groups: (1) patients with acute and chronic hepatitis; (2) multiple transfused patients and intravenous drug users; and (3) blood donors [1,3–8]. Several very recent publications demonstrated a rather high prevalence of GBV-C/HGV infection in patients on maintenance haemodialysis [5,9–12].

The clinical significance of GBV-C/HGV infection remains uncertain. Although viral RNA was detected in a fraction of patients with fulminant, acute, and chronic hepatitis, most of the existing data on the causative role of GBV-C/HGV infection in hepatitis non-A,E are controversial [3–9,13].

Reverse transcriptase-polymerase chain reaction (RT-PCR) remains the sole diagnostic method for both acute and chronic GBV-C/HGV infection. In contrast to HCV infection, in individuals with GBV-C/HGV viraemia no antibodies to viral structural or non-structural proteins can consistently be detected. The only serological markers, antibodies to the putative envelope protein 2 (anti-E2), appear at the convalescent phase and are considered as an indicator of past GBV-C/HGV infection [14,15]. In most cases viral RNA and anti-E2 are mutually exclusive. In this respect GBV-C/HGV is clearly different from HCV, but shows a similar serological pattern to another member of Flaviviridae group, tick-borne encephalitis virus (TBEV). The humoral immune response in TBEV infection is mostly directed against the viral envelope protein and becomes evident only after disappearance of the viral RNA from serum.
In this study we used RT-PCR and an enzyme immunoassay to detect viral RNA and antiviral antibodies (anti-E2) respectively, in sera of patients treated with haemodialysis (HD) or continuous ambulatory peritoneal dialysis (CAPD), as well as in sera of renal transplant (RT) recipients. We also searched for a possible association between these markers and liver disease in GBV-C/HGV-infected patients. Information on the prevalence and characteristics of the GBV-C/HGV infection in patients with renal failure should help us to minimize the risks of being infected both for patients and staff and to prevent the spread of the virus in the haemodialysis and transplantation units.

Subjects and methods

Patients

The study involved 148 patients with end-stage kidney disease treated at the department of nephrology, Essen University Clinics. Thirty-one patients (22 male, aged $53 \pm 15$ years) were treated with haemodialysis (HD group), 25 (16 male, 47 ± 15 years) with continuous ambulatory peritoneal dialysis (CAPD group), and the remaining 92 (46 male 49 ± 13 years) were subjected to renal transplantation (RT group). Patients who underwent renal transplantation were excluded from HD and CAPD groups. The patients had been on haemodialysis and peritoneal dialysis for 2–252 months (median 25) and for 6–288 months (median 38) respectively. The time of follow-up after renal transplantation varied between 1 and 204 months (median 50). The renal graft recipients continuously received immunosuppressive therapy consisting of prednisolone and azathioprine with or without cyclosporin. Alanine aminotransferase (ALT) levels were determined at monthly intervals. Additionally, two control groups comprising randomly chosen 128 multiple-organ donors and 368 volunteer blood donors were enrolled in the study.

RT-PCR and nucleotide sequencing

Serum samples were tested for HCV RNA by ‘AmpliCtor’ (Roche Diagnostic System). GBV-C/HGV RNA was determined by RT-PCR as described earlier [16] with a few modifications. RNA was extracted from 140 μl of serum with the QIAamp Viral RNA Kit (QIAGEN) and was converted to the complementary DNA (cDNA) with an antisense primer (YK-877 5'-ACCGACACCTTAGATCCCAGGCCC). Reverse-transcribed cDNA was subjected to the first round of 35-cycle PCR with primers YK-877 and YK-874 (5'-CTGATGTTGCTAGCCTGTGTGAGA). Second round of 35-cycle PCR was run with primers YK-876 (5'-CCCTACAGTCTTATGGTTCTTC) and YK-1183 (5'-CAGAAAACTACAGGCTTATGGTGA). All primer sequences were derived from the NS5 region of HGV genome. Each cycle included denaturation at 94 °C for 1 min, annealing at 60 °C for 1 min, and extension at 72 °C for 2 min. PCR products were separated by agarose gel electrophoresis and visualized by ethidium bromide staining. Recently we have participated in the first German national multicentre trial of GBV-C/HGV RNA detection methods. RT-PCR variant used by our group [17] demonstrated 100% positivity in terms of sensitivity and specificity (E. Schreier and A. Kekule, unpublished). PCR fragments were gel purified with Qiaquick gel extraction kit (Qiagen) and subjected to direct sequencing from both directions using the Dye Terminator Cycle Sequencing kit (Perkin Elmer).

Phylogenetic analysis

Newly obtained sequences were compared with GBV-C/HGV sequences taken from the GenBank and reported earlier [6,16] by means of the phylogenetic analysis using programs DNADIST and NEIGHBOR from the package PHYLIP, version 3.5c (Felsenstein, J. 1993. Department of Genetics, University of Washington, Seattle, USA). The evaluation of the significance of the grouping was assessed by the bootstrap analysis (100 replicates) using the program SEQBOOT.

Detection of serological markers of GBV-C/HGV, HCV, and HBV infections and ALT levels

Serum samples were analysed for anti-HCV and HBsAg with enzyme-linked immunosorbent assay (Sorin Biomedica). Antibodies to the envelope E2 protein of GBV-C/HGV (anti-E2) were detected with the immunoassay kit ‘Anti-HGen’ kindly provided by Boehringer Mannheim. ALT levels were determined on a multiparametric analyser using the ‘FCC method at 25 °C.

Statistical analysis

Results were analysed with the chi-square test for categorical variables and by the Mann–Whitney test for non-parametric qualitative data. P values of <0.05 were considered as a significant difference between groups.

Results

Table 1 demonstrates the prevalence of GBV-C/HGV, HCV, and HBV markers in three groups of patients with renal failure. A statistical trend was noted for a higher rate of the GBV-C/HGV RNA detection in HD (9.7%) and RT (15.2%) patients in comparison with organ donors (5.5%) (P = 0.056; see note to Table 1). However, upon a comparison of the GBV-C/HGV RNA frequencies in each of these patients groups with that among donors, only the difference between GBV-C/HGV RNA prevalence in RT patients (15.2%) and in organ donors (5.5%) was found to be significant (P = 0.028). Anti-E2 antibodies were detected with almost the same frequency in all groups, including organ and blood donors. None of the infected individuals had simultaneously GBV-C/HGV RNA and anti-E2. Overall, 36 of 148 patients with renal failure (24.3%) and 21 of 128 organ donors (16.4%) had evidence of ongoing or past GBV-C/HGV infection. GBV-C/HGV RNA was not detected in CAPD patients and 20% of them were found to be positive for anti-E2. The relatively low number of CAPD patients, however, has to be taken into account in the analysis of these data. None of the GBV-C/HGV RNA-positive patients had any evidence of HCV infection and three patients had both GBV-C/HGV RNA and HBsAg.
Demographic, biochemical, and virological features were compared between patients who were positive for GBV-C/HGV RNA in the serum and those who were not (Table 2). There were no significant differences in sex, age, prevalence of HCV RNA, anti-HCV, HBsAg, ALT levels, or history of blood transfusions between these groups. It should be noted, however, that a history of blood transfusions was reported by all patients with GBV-C/HGV RNA. Time on haemodialysis was longer in patients infected with GBV-C/HGV (median 68; range 25–138 months) compared to non-infected patients (median 23; range 2–288 months). The difference was statistically significant (P<0.05). Similar analysis for patients with and without anti-E2 revealed no statistically significant differences for these groups with regard to the features studied (Table 2).

DNA amplificates from 14 GBV-C/HGV-positive patients were purified and subjected to direct sequencing. Phylogenetic analysis of the resulting sequences together with a set of sequences from the GenBank for a higher prevalence of GBV-DNA amplificates from 14 GBV-C/HGV RNA in the serum and those who were C/HGV RNA Anti-E2 HCV RNA Anti-HCV HBsAg

1. Patients with renal failure: 148 17 (11.5%) 19 (12.8%) 12 (8.1%) 17 (11.5%) 9 (6.1%)
   (a) Haemodialysis patients (HD) 31 3 (9.7%) 4 (12.9%) 0 2 (6.5%) 3 (9.7%)
   (b) Patients on peritoneal dialysis (CAPD) 25 0 5 (20.0%) 2 (8.0%) 2 (8.0%) 0
   (c) Renal transplant recipients (RT) 92 14 (15.2%) 10 (10.9%) 10 (10.9%) 13 (14.1%) 6 (6.5%)

2. Multiple organ donors (OD) 128 7 (5.5%) 14 (11.1%) nt nt nt
3. Blood donors (BD) 368 nt 40 (10.9%) nt nt nt

None of the observed differences was statistically significant. GBV-C/HGV RNA rate 9.7, 0, 5.5% in HD, CAPD, RT patients, and OD respectively; chi-square 3.604 (2 df) P=0.065. Anti-E2 frequency 12.9, 20.0, 10.9% in HD, CAPD, RT patients, and in OD and BD respectively; chi-square 1.535 (4 df) P=0.82. HCV RNA frequency 0, 8, 10.9% in HD, CAPD, and RT patients respectively; chi-square 3.294 (2 df) P=0.193. Anti-HCV frequency 6.5, 8.0, 14.1% in HD, CAPD, and RT patients respectively; chi-square 1.38 (2 df) P=0.5. HBsAg rate 9.7, 0, 6.5% in HD, CAPD, and RT patients respectively; chi-square 2.149 (2 df) P=0.341.

Discussion

At present only two methods to detect markers of GBV-C/HGV infection are available. One is RT-PCR, which detects viral RNA in serum, and therefore is used for identification of acute and chronic infections. Another is the serological enzyme immunoassay (ELISA) for antibodies to the E2 protein of viral particles. These antibodies are indicators of a past GBV-C/HGV infection [14,15]. Only the combination of these two techniques provide the most accurate assessment of the occurrence and prevalence of GBV-C/HGV infection in a particular population group.

Using the RT-PCR we demonstrated a tendency for a higher prevalence of GBV-C/HGV RNA in patients with renal failure (11.5%) and significantly higher rate of this RNA in RT patients (15.2%) than in a control group of organ donors (5.5%). These data, as well as the reported rates of GBV-C/HGV RNA in German blood donors (2.5–4.5%) [13] suggest that patients with renal failure and in particularly RT patients are at increased risk of GBV/C/HGV infection.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>GBV-C/HGV RNA</th>
<th>Anti-E2</th>
<th>HCV RNA</th>
<th>Anti-HCV</th>
<th>HBsAg</th>
</tr>
</thead>
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<td>0</td>
<td>2 (6.5%)</td>
<td>3 (9.7%)</td>
</tr>
<tr>
<td>(b) Patients on peritoneal dialysis (CAPD)</td>
<td>25</td>
<td>0</td>
<td>5 (20.0%)</td>
<td>2 (8.0%)</td>
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<td>0</td>
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<tr>
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<td>13 (14.1%)</td>
<td>6 (6.5%)</td>
</tr>
<tr>
<td>2. Multiple organ donors (OD)</td>
<td>128</td>
<td>7 (5.5%)</td>
<td>14 (11.1%)</td>
<td>nt</td>
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<td>3. Blood donors (BD)</td>
<td>368</td>
<td>nt</td>
<td>40 (10.9%)</td>
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</table>

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Table 1. Prevalence of markers of GBV-C/HGV and HCV infections in patients with renal failure and in organ and blood donors

<table>
<thead>
<tr>
<th>Group</th>
<th>Detection of GBV-C/HGV RNA</th>
<th>Differences</th>
<th>Detection of anti-E2</th>
<th>Differences</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive (n=17)</td>
<td>Negative (n=131)</td>
<td>Positive (n=19)</td>
<td>Negative (n=129)</td>
</tr>
<tr>
<td>Male</td>
<td>14 (82%)</td>
<td>70 (53%)</td>
<td>NS**</td>
<td>10 (53%)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>45±13*</td>
<td>49±14</td>
<td>NS</td>
<td>49±15</td>
</tr>
<tr>
<td>HCV RNA</td>
<td>0</td>
<td>12 (9.2%)</td>
<td>NS</td>
<td>2 (10.6%)</td>
</tr>
<tr>
<td>Anti-HCV</td>
<td>0</td>
<td>17 (13%)</td>
<td>NS</td>
<td>4 (21%)</td>
</tr>
<tr>
<td>HBsAg</td>
<td>3 (17.6%)</td>
<td>6 (46.6%)</td>
<td>NS</td>
<td>1 (5%)</td>
</tr>
<tr>
<td>ALT (IU/l)</td>
<td>10±7</td>
<td>8±4</td>
<td>NS</td>
<td>8±3</td>
</tr>
<tr>
<td>Duration of dialysis (months)</td>
<td>68 (25–138)**</td>
<td>23 (2–288)</td>
<td>S****</td>
<td>37 (11–252)</td>
</tr>
<tr>
<td>History of blood transfusion</td>
<td>17 (100%)</td>
<td>89 (68%)</td>
<td>NS</td>
<td>14 (74%)</td>
</tr>
</tbody>
</table>

* Mean±SD; **Median (range); ***NS, not significant; ****S, significant (P<0.05).
GBV-C/HGV infection in renal dialysis and transplant patients

and in immunosuppressed renal graft recipients. The resolution of the GBV-C/HGV infection in such patients might be a relatively rare event. Several recent publications provide some evidences supporting this hypothesis. First, Neilson et al., 1996 [17], demonstrated higher prevalence of GBV-C/HGV RNA in patients who were profoundly immunosuppressed due to bone marrow transplantation. Masuko et al., 1996 [5] followed eight GBV-C/HGV RNA-positive haemodialysis patients for 7–14 years and demonstrated that only one of these patients had lost viral RNA after 10 years. On the other hand, the resolution rates of the GBV-C/HGV infection in non-immunosuppressed individuals seem to be much lower. Thus, Alter et al., 1997 [4] reported a clearance of GBV-C/HGV within 3 years in 3 of 8 subjects infected as a result of haemotransfusion. According to another paper [8], the persistence rate of the GBV-C/HGV infection acquired by blood transfusion by non-immunosuppressed patients was less than 40%; 8 of 22 infected subjects have lost viral RNA during the follow-up from 9 months to 8 years after transfusion.

We were unable to identify any particular risk factor associated with the presence of anti-E2 in patients with renal failure. On the other hand, patients with the GBV-C/HGV RNA had been on haemodialysis for a significantly longer time and all of them had a history of blood transfusions compared to patients without viral RNA. This finding seems to supplement the recent data of Masuko et al., 1996 [5], who demonstrated that some of 16 GBV-C/HGV RNA-positive HD patients were infected by haemotransfusions and some most probably by other means of transmission.

GBV-C/HGV RNA positivity rates in our groups of patients with renal failure are slightly higher than those reported for HD region (positions 7271–7624, according to the sequence of the GBV-C prototype isolate; GenBank accession number (a.n.) U36380). The sequences from the present study are indicated by asterisks. Three sequences of the GBV-C, HGV1, and HGV2 prototype isolates have the GenBank a.n. U36380, U44402, and U45966. Other sequences were taken from [6,16].

ports the notion [14,15] that anti-E2 are associated with a loss of viraemia. Thus GBV-C/HGV and HCV infections are characterized by different virological and serological profiles; in the majority of HCV infected individuals viral RNA is present and persists in the presence of anti-HCV antibodies.

The prevalence of anti-E2 in patients with renal failure and in organ and blood donors was almost the same. On the first glance these results suggest the similar rates of exposure to GBV-C/HGV in all groups studied, and therefore contradict both our results and all available data [5,9–12] on the higher rates of GBV-C/HGV infection in patients with renal failure than in general population. One of the possible explanation of this controversy might be that the establishment of the GBV-C/HGV infection and its chronic outcome are probably more frequent in dialysis patients, generally characterized by impairment of their immune response,
lished [6,16], we were unable to detect any major grouping, significance of which could have been confirmed by a bootstrap analysis (Figure 1). These data indicate that the GBV-C/HGV strains from patients with renal failure most probably do not differ from other viral isolates, including those which had been obtained from blood donors, multiple transfused patients, intravenous drug addicts, and haemophiliacs. Recently we have amplified GBV-C/HGV sequences from two of our patients with primers derived from the 5' NCR. Phylogenetic analysis of these sequences demonstrated their relatedness to European/American variant of GBV-C/HGV but not to the African or Asiatic types (S.Viazov, unpublished). In addition, GBV-C/HGV sequences from our study did not segregate into any closely related pairs (Figure 1). This result can be considered as an evidence against our patients being infected from the same source or patient to patient transmission of GBV-C/HGV.

One of the most intriguing question of GBV-C/HGV research is whether this virus is associated with any disease of humans, and in particular with hepatitis. None of our 17 patients with GBV-C/HGV RNA or 19 patients with anti-E2 antibodies were icteric or had any biochemical or clinical signs of liver disease. It should be noted, however, that serum amino-transferases levels are usually decreased in chronic dialysis patients, and a proposal to lower the cut-off value of serum transaminases for detection of liver disease in HD patients has recently been published [20]. Thus our data cannot exclude a possibility of underlying viral infection of hepatocytes at least in some of our patients. Prospective clinical and epidemiological studies are needed to determine the long-term outcome of GBV-C/HGV infection in patients with renal failure.

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References


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