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GBV-C/HGV in hemodialysis patients: Anti-E2 antibodies and GBV-C/HGV-RNA in serum and peripheral blood mononuclear cells

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GBV-C/HGV in hemodialysis patients: Anti-E2 antibodies and GBV-C/HGV-RNA in serum and peripheral blood mononuclear cells. Hepatitis G virus (GBV-C/HGV), a recently identified RNA virus adds to the risk of parenteral transmitted viral infections in hemodialysis patients. We studied the prevalence of GBV-C/HGV-RNA in serum and peripheral blood mononuclear cells (PMNC) by reverse transcription-polymerase chain reaction (RT-PCR) and determined antibodies against the envelope protein E2 of GBV-C/HGV by ELISA. A total of 119 dialysis patients were studied. GBV-C/HGV-RNA was found in 16 of 119 patients (13%) as compared with 2% of healthy controls ($P = 0.014$). Two of the 16 GBV-C/HGV-RNA+ patients were co-infected with HCV, and none was positive for HBV-DNA. In 38% of serum GBV-C/HGV-RNA+ patients GBV-C/HGV-RNA was also detected in PMNC. In addition, GBV-C/HGV-RNA was identified in PMNC of 2 patients negative for GBV-C/HGV-RNA in serum. Twenty-four patients had anti-E2 antibodies in serum (20%), but were GBV-C/HGV-RNA−. In addition, two of the 16 GBV-C/HGV-RNA+ patients were concomitantly positive for anti-E2 antibodies. Only one of the 16 GBV-C/HGV infected patients had elevated aminotransferases; this patient was co-infected with hepatitis C virus. GBV-C/HGV-RNA positivity was independent on duration of hemodialysis, but GBV-C/HGV-RNA+ patients had received more units of blood in the past. Combined data of past contact, as assessed by anti-E2 antibodies, and present infection, documented by GBV-C/HGV-RNA, indicate a high overall exposure to GBV-C/HGV in dialysis patients.

Patients undergoing chronic intermittent hemodialysis carry a variable risk for acquiring parenterally transmitted infections such as hepatitis B (HBV) or hepatitis C (HCV) [1]. With HBV infection still present but less prevalent in that group (5.6%) [1], the growing impact of HCV infection has been documented in several surveys showing seroprevalence rates from 8% to 55% [2, 3].

Recently, a new RNA virus belonging to the family of Flaviviridae has been identified by two groups [4, 5]. This virus has been shown to be associated with hepatitis and transmitted parenterally and is now commonly referred as GBV-C/HGV hepatitis virus [6]. GBV-C/HGV has been shown to be associated with infections of other parenterally transmitted viruses such as HBV or HCV [5, 7]. In addition to being present in serum HGV-RNA has also been found in PMNC [8]. In case of HBV and HCV, we reported previously that investigating PMNC for the presence of viral genomes can be useful in the positive identification of additional potential infectious patients [9].

To study the infection of GBV-C/HGV in hemodialysis patients, we used reverse transcription polymerase chain reaction (RT-PCR) to assess the prevalence of GBV-C/HGV-RNA in serum. As GBV-C/HGV has been detected in lymphocytes we also looked for the presence of GBV-C/HGV-RNA in PMNC. In addition, we determined antibodies against the envelope protein of GBV-C/HGV (anti-E2) by ELISA as a marker of resolved infection with GBV-C/HGV. We report that GBV-C/HGV-RNA can be found in serum of 13% of hemodialysis patients and that in some infected patients GBV-C/HGV-RNA can only be found in PMNC, but not in serum. In addition, 20% of the patients had had past contact with GBV-C/HGV, as judged by the presence of anti-E2.

METHODS

Patients

We investigated 119 patients (69 male, 50 female, mean 53 ± 17 years) undergoing chronic hemodialysis at the dialysis units of the Universitätsklinik für Innere Medizin III, University of Vienna. Mean duration of dialysis was 35 ± 32 months. Renal disorders resulting in end-stage kidney disease were glomerulonephritis (27 patients), diabetic nephropathy (18 patients), analgesic nephropathy (7 patients), interstitial nephritis (15 patients) and miscellaneous causes (16 patients). The etiology of end-stage renal disease could not be established in 36 patients. In regular intervals (every 3 months) infection with hepatitis virus B or C was checked by serologic methods. Serologic determinations of HBV markers (HBsAg, anti-HBs, anti-HBc, HBeAg, anti-HBe; Abbott Laboratories) and of HCV markers (anti-HCV antibody, 3rd generation; Abbott Laboratories) were performed by the ELISA method

Key words: hepatitis G and C virus, RNA virus, dialysis, infections, GBV-C/HGV hepatitis virus.

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Table 1. Clinical data and liver function tests of patients undergoing chronic hemodialysis

	Patients with evidence for present GBV-C/HGV infection	Patients without evidence for present GBV-C/HGV infection		
	GBV-C/HGV-RNA+	Anti-E2+, GBV-C/HGV-RNA−	Anti-E2−, GBV-C/HGV-RNA−	*P*
Number of patients	16	24	79	
Gender *male, female*	7m, 9f	15m, 9f	62m, 41f	NS
Age *years*	50 ± 17	53 ± 14	54 ± 18	NS
Duration of hemodialysis *months*	46 ± 43	30 ± 27	35 ± 31	NS
AST *U/liter*	10 ± 15	9 ± 10	9 ± 6	NS
ALT *U/liter*	10 ± 11	9 ± 7	10 ± 7	NS
γGT *U/liter*	23 ± 30	51 ± 122	34 ± 65	NS
AP *U/liter*	157 ± 79	148 ± 79	142 ± 100	NS
CHE *kU/liter*	3.6 ± 1.4	4.3 ± 1.2	3.8 ± 1.2	NS
Bilirubin *mg/dl*	0.5 ± 0.2	0.5 ± 0.2	0.5 ± 0.2	NS
Albumin *g/liter*	41 ± 4	42 ± 4	42 ± 5	NS
Number of patients transfused	11 (69%)	13 (54%)	31 (39%)	NS
Number of blood units given	9 ± 16	8 ± 21	4 ± 10	< 0.05

using commercially available kits. None of the patients was HBsAg+, and 26 patients had markers of past HBV infection (antiHBc+). Seventy-eight patients were antiHBs+, either by natural infection or as a result of vaccination with a recombinant hepatitis B vaccine (Engerix; Smith Kline Beecham); 41 patients were non-responders to vaccination. Seventeen patients had positive anti-HCV antibodies, and in 15 of them HCV-RNA could be detected by PCR. Laboratory data of the patients included in this study are given in Table 1.

Controls

Ninety-three healthy individuals recruited from a screening program for thyroid diseases served as controls.

Processing of blood samples

Serum samples and heparinized peripheral venous blood was collected at the same time from each patient using the vacutainer system (Becton Dickinson). All sample handling was performed under sterile conditions in a laminar air flow system in a dedicated room separated from the area where PCR reactions were set up. The recommended precautions to avoid contamination by PCR product carryover and false-positive results were followed [10]. Peripheral blood mononuclear cells (PMNC) were separated by buoyant density gradient centrifugation using Ficoll-Hypaque (Pharmacia). The mononuclear cell fraction was aspirated and washed six times with 30 ml of sterile 0.9% saline. This extensive washing step has been shown in our laboratory to remove serum-associated viral contamination from the PMNC fraction; PCR from an aliquot of the last washing fluid was performed in each patient and gave consistently negative results. Purified and washed PMNC were counted, 2 to 5×10^6 PMNC were lysed in 500 ml 4 M guanidium isothiocyanate/1% 2-mercaptoethanol, and stored at −70°C together with serum samples and an aliquot of the last washing fluid.

Preparation of DNA and RNA for polymerase chain reaction

Preparation of DNA for HBV-PCR and RNA for HCV-PCR and GBV-C/HGV-PCR from serum samples, PMNC and last washing fluid of PMNC was done as described previously [9]. Oligonucleotide primers for HBV-DNA and HCV-RNA and cycling conditions for nested PCR for HBV and HCV were the same as described [9].

Reverse transcription was performed with RNA from 7 μl serum or last washing fluid or from 1×10^5 PMNC, 5 × reverse transcription buffer (Cetus, USA) 200 nmol Dithiothreitol (Gibco BRL, Life Technologies, Gaithersburg, PA, USA), 10 pmol dNTP (Cetus) each, 100 pmol random hexamer primer [$pd(N)_6$, Pharmacia, Uppsala, Sweden], 20 U RNAsin (Promega) and 200 U M-MLV RT (Moloney murine leukemia virus reverse transcriptase, Gibco, NY, USA) in 20 μl volume for one hour at 37°C. After a denaturation step at 99°C for 10 minutes 30 μl of master mix for the first round PCR containing 10 × PCR buffer, 5 pmol dNTP each, 40 pmol each of outer primers (GB-C-sl and GB-C-al) [4] and 1 U *Taq* polymerase (Cetus) was added. The cycling protocol was as follows: two minutes 95°C, then 35 cycles of 15 seconds 94°C, 30 seconds 50°C and 30 seconds 72°C. One microliter of the first PCR reaction was then transferred to a second tube containing 10 × PCR buffer, 4 pmol dNTP each, 40 pmol of inner primers each (GB-C.5-sl and GB-C.5-al) [4] and 1 U *Taq* polymerase in a total volume of 100 μl. The cycling protocol was the same as for the first PCR. The amplified DNA product of 127 bp length was analyzed on a 1% agarose gel after staining with ethidium bromide. The specifity of the product was verified by hybridization to an internal digoxigenin-labeled oligonucleotide probe (5′-AGG-CGG-AGT-GCG-AGA-GAT-TGG-CCG-GC-3′) after transfer to a nylon membrane. In every PCR run, two reagent controls, two positive and two negative samples were included. All samples were run in duplicate from the beginning of the extraction procedure until gel analysis, and only congruent results were accepted.

Anti-E2 antibodies

A commercially available ELISA (Enzymun-Test Anti-HGenv, Boehringer Mannheim, Germany) detecting serum antibodies against the envelope antigen E2 of GBV-C/HGV was used [11].

Table 2. Prevalence of GBV-C/HGV in serum in relation to presence of HBV and HCV in serum and of GBV-C/HGV-RNA in PMNC

	GBV-C/HGV serum +	GBV-C/HGV serum −	Total
HBV serum +	0	2	2
HBV serum −	16	101	117
Total	16	103	119
HCV serum +	2	13	15
HCV serum −	14	90	104
Total	16	103	119
PMNC +	6	2	8
PMNC −	10	101	111
Total	16	103	119

RESULTS

GBV-C/HGV-RNA in serum

We found GBV-C/HGV-RNA in 16 of 119 patients on chronic hemodialysis (13%); this was significantly higher than the 2 positive individuals found in 93 healthy controls (2%; $P = 0.014$; Table 2). None of the GBV-C/HGV-RNA+ patients was co-infected with HBV as judged by HBsAg and HBV-DNA (Table 1); 2 of the 16 GBV-C/HGV-RNA+ patients (13%) were co-infected with HCV (anti-HCV+, HCV-RNA+; Table 2).

GBV-C/HGV-RNA in peripheral blood mononuclear cells

GBV-C/HGV-RNA was detected in PMNC of 6 out of 16 patients with GBV-C/HGV-RNA present in serum (38%; Table 2). In addition, we identified 2 serum GBV-C/HGV-RNA− patients in whom GBV-C/HGV-RNA could be demonstrated in PMNC only.

Anti-E2 envelope protein

In 26 patients (22%) anti-E2 were found in serum, and in 24 of those patients GBV-C/HGV-RNA was negative. Two other patients were concomitantly positive for GBV-C/HGV-RNA and anti-E2 in the group of 16 GBV-C/HGV-RNA+ patients. Two additional patients, who were GBV-C/HGV-RNA+ in PMNC but not in serum, were negative for anti-E2. Seventy-nine patients were negative for both GBV-C/HGV-RNA and anti-E2. The prevalence of anti-E2 in hemodialysis patients (26 of 119 patients; 22%) was not different as compared to healthy controls (12 of 93 healthy controls; 13%; $P = 0.13$).

Clinical correlations

We compared clinical features of patients positive for GBV-C/HGV-RNA in serum with those of patients without evidence for GBV-C/HGV-infection (Table 1). We found no difference in the mean duration of hemodialysis between those two groups. Patients with past or present contact to GBV-C/HGV had received more units of blood than patients without evidence for GBV-C/HGV infection ($P < 0.05$; Table 1). Only one of the GBV-C/HGV-RNA+ patients had elevated aminotransferases; however, this patient was also co-infected with HCV. Two additional HCV+ but HGV− patients had moderately elevated ALT levels. There was no significant difference in markers of cholestasis, such as alkaline phosphatase and γGT or in markers of liver synthesis (albumin and pseudocholinesterase) or bilirubin between dialysis patients positive for GBV-C/HGV-RNA or positive for anti-E2 or negative for markers of GBV-C/HGV infection. No other laboratory differences were found between these three groups. We found a significant association of anti-HCV positivity with markers of past or present GBV-C/HGV infection (GBV-C/HGV-RNA+ or anti-E2+) (χ^2-test, $P = 0.003$); however, there was no association of past contact to hepatitis B virus (anti-HBc+) with past or present contact to GBV-C/HGV (χ^2-test, $P = 0.554$).

DISCUSSION

In the present study we showed a high prevalence of exposure of patients on chronic hemodialysis to GBV-C/HGV: GBV-C/HGV-RNA as well as anti-E2, indicating past contact to GBV-C/HGV, were higher than found in healthy control individuals. In addition, detection of GBV-C/HGV-RNA in PMNC disclosed some patients as infected who had neither GBV-C/HGV-RNA nor anti-E2 in serum.

GBV-C/HGV is assumed to be parenterally transmitted [12] and patients on chronic hemodialysis constitute a group at risk for such infection. An increased prevalence for GBV-C/HGV in patients on chronic hemodialysis as compared to local control population has been demonstrated by several groups [7, 13–18]. This increased prevalence was also observed in our patient population, in which 13% were GBV-C/HGV-RNA+ as compared to 2% of healthy local controls. In this respect GBV-C/HGV is very similar to other parenterally transmitted hepatitis viruses, such as HBV or HCV, which have also an increased prevalence in dialysis patients [1, 9, 19].

There seems to be a wide range of GBV-C/HGV infection in hemodialysis patients among different countries: available data from Indonesia (55%) [15] and France (57%) [14] show a high prevalence of GBV-C/HGV-RNA, whereas it was reported to be low in Japan (3 to 10%) [13, 16] and Germany (5 to 7%) [7, 17]. Our data from Austria and recently published data from Italy (19%) [18] indicate a similar relatively low prevalence of GBV-C/HGV-RNA as opposed to France or Indonesia. Similar country specific differences in prevalence have also been observed for hepatitis B and C [1, 20]. In Indonesia the high prevalence of GBV-C/HGV-RNA in patients on chronic hemodialysis corresponded to a high prevalence of HCV-RNA (76%) [21] and HBsAg (7%) [21]. Countries with low prevalence of GBV-C/HGV-RNA such as Japan (10%) also had a relatively low prevalence of anti-HCV (38%) [22] and HBsAg (1.6%) [23] in dialysis patients. Similarly, in our patient population there was a relatively low prevalence of GBV-C/HGV-RNA (13%), which corresponded to a low prevalence of HCV-RNA (15%) and HBsAg (0%).

GBV-C/HGV genome can be detected in PMNC [8]. In this respect GBV-C/HGV is similar to HBV and HCV for which transcription and replication in PMNC has been described [24, 25]. We found GBV-C/HGV-RNA in 8 of 119 patients on chronic hemodialysis (7%), which is similar to the positivity rate of PMNC for HBV-DNA (6%) or HCV-RNA (9%) as described previously by our group [9]. More than a third of the patients positive for GBV-C/HGV-RNA in serum (28%) also had GBV-C/HGV-RNA in their PMNC. In addition, we identified 2 patients who were negative for GBV-C/HGV-RNA in serum in whom GBV-C/HGV-RNA could be demonstrated in their PMNC only; those patients were also anti-E2 negative. Thus, testing for GBV-C/HGV-RNA in PMNC in addition to serum is a more sensitive

approach than serum testing alone. By searching for GBV-C/HGV in PMNC, occult infection with this virus might be uncovered.

Recently, an antibody test system to detect antibodies to the envelope protein (E2) of GBV-C/HGV has been described [11]. In general anti-E2+ patients have lost GBV-C/HGV-RNA from serum and the presence of anti-E2 is thought to indicate resolved infection. Thus, the combined detection of GBV-C/HGV-RNA and anti-E2 is necessary to define the overall exposure to GBV-C/HGV. Only 22% of our patients were positive for anti-E2. This is less than the 41% reported for drug users [11], but more than 8.5% found by others in dialysis patients [17]. We confirm in this patient population the observation that the presence of anti-E2 is associated with the loss of detectable GBV-C/HGV-RNA. Only 2 of 26 patients with anti-E2 had GBV-C/HGV-RNA demonstrable in serum. The percentage of patients positive for both GBV-C/HGV-RNA and anti-E2 (7.6%) compares well to that reported by others [11, 17], but contrasts to a report describing GBV-C/HGV-RNA and anti-E2 antibodies as mutually exclusive [26]. Combining the numbers of GBV-C/HGV-RNA+ with that of anti-E2+ patients gives a more accurate picture of exposure to hepatitis G virus in this population: 34% of our patients on chronic hemodialysis had contact to the virus. This is considerably more than reported in another dialysis population in Central Europe [17] for which the combined prevalence of GBV-C/HGV-markers was only 7.8%. That difference in prevalence in GBV-C/HGV-markers indicates a high variability in exposure to the virus even in nearby geographic areas as previously reported for HBV and HCV infection [23] and makes it necessary to determine the local prevalence of GBV-C/HGV. The prevalence of anti-E2 among dialysis patients was not significantly different from that among healthy population, but the prevalence of GBV-C/HGV-RNA was significantly higher among dialysis patients. The impaired immune response in dialysis patients [27] could be the reason for the higher percentage of active HGV infections.

In accordance with the reports of others [13, 18], GBV-C/HGV positive patients in our chronic hemodialysis program had no evidence for liver damage as judged by elevated aminotransferases. Only one of our GBV-C/HGV-RNA+ patients had increased AST levels, which we attributed to his co-infection with HCV. Furthermore, we could not demonstrate an association of the duration of hemodialysis with GBV-C/HGV-status (GBV-C/HGV-RNA+ or anti-E2+). However, as a possible hint to the mode of transmission of GBV-C/HGV we and others [14] could demonstrate that the group of patients with GBV-C/HGV contact had received significantly more units of blood. This mode of transmission is further suggested by a significant association between contact to HCV and GBV-C/HGV.

In summary, patients on chronic hemodialysis have a high prevalence of markers for present or past infection with hepatitis G virus. Additional determination of anti-E2 allows us to draw a more complete picture of contact to GBV-C/HGV than testing for GBV-C/HGV-RNA alone. GBV-C/HGV infection does not induce elevation of aminotransferases in this patient group, and no specific clinical features distinguishing GBV-C/HGV-RNA+ from GBV-C/HGV-RNA− patients could be identified. The clinical importance of this infection remains to be established.

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