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Hepatitis G Virus Infection in Hemodialysis and Continuous Ambulatory Peritoneal Dialysis Patients

Hyunjin Noh[1], Shin Wook Kang[1], Seung Hyuk Choi[1], Sug Kyun Shin[1], Bo Jeung Seo[1], In Hee Lee[1], Kyu Hun Choi[1], Dae Suk Han[1], Hyon Suk Kim[2], and Ho Yung Lee[1]

To determine the prevalence and clinical relevance of HGV infection in dialysis patients, we performed a cross-sectional study of 61 HD patients and 79 Continuous Ambulatory Peritoneal Dialysis(CAPD) patients. HGV-RNA was identified by reverse-transcription (RT) polymerase chain reaction (PCR) assay with primers from the 5'-untranslated region of the viral genome. The prevalence of HGV infection was similar in HD and CAPD patients (9.8% vs. 12.7%), while that of HCV infection was significantly higher in HD patients compared to CAPD patients (16.4% vs. 1.3%, $p<0.05$). The mean age (49.2 ± 13.4 vs. 46.7 ± 13.0 years), male to female ratio (2.4:1 vs. 1.3:1), history of transfusion (62.3% vs. 49.4%), history of hepatitis (27.9% vs. 26.6%), mean ALT level during the previous 6 months (22.4 ± 37.9 vs. 14.0 ± 7.4 IU/L), and the prevalence of HBsAg (8.2% vs. 6.3%) showed no difference between HD and CAPD patients. In both HD and CAPD patients, the presence of HGV RNA was not related to age, sex, duration of dialysis, history of transfusion, history of hepatitis, or to the presence of HBV or HCV markers. There was no significant difference in the clinical and biochemical data between patients with isolated HGV infection (n=12) and patients without viremia (n=106). The clinical features of patients coinfected with HGV and HBV (n=2), or HGV and HCV (n=2) seemed to be similar to those of patients with isolated HBV (n=8) or HCV (n=9) infection. In conclusion, the prevalence of HGV infection was not different between HD and CAPD patients, and HGV infections did not seem to be associated with clinically significant hepatitis. The routes of HGV transmission, other than transfusion or contamination during HD procedure, were suspected.

Key Words: Hepatitis G virus, hemodialysis, CAPD

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[1]Department of Internal Medicine, Institute of Kidney Disease, [2]Department of Clinical Pathology, Yonsei University College of Medicine, Seoul, Korea
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Address reprint request to Dr. H.J. Noh, Department of Internal Medicine, Yonsei University College of Medicine, C.P.O. Box 8044, Seoul 120-752, Korea, e-mail: noh@medikorea.net

Patients undergoing dialysis potentially have an increased risk of infection with parenterally-transmitted viral agents due to an impaired host immune response and multiple transfusion requirements (Goldblum and Reed, 1980). Especially in hemodialysis (HD) patients, transmission by contamination during dialysis cannot be ruled out. Viral hepatitis has been recognized as a relevant problem for HD patients because 1.9% of all deaths among HD patients are regarded as sequelae of viral hepatitis (Jakobs *et al.* 1977). Since HBV infection has been reduced by

active immunization, periodic tests for viral markers and improved disinfectant procedures, HCV infection has become the most common type of hepatitis among patients on maintenance HD (Galbraith *et al.* 1979), and is thought to be a major cause of non-A non-B hepatitis (Alter *et al.* 1986; Zeldis *et al.* 1990). However, about 10~15% of patients with parenterally-transmitted non-A non-B hepatitis have no evidence of HCV infection after extensive evaluation (Alter and Bradley, 1995). These patients also have no evidence of infection with HDV or HEV, therefore they can be classified as having non-A-E hepatitis.

Recently, two isolates of a new virus, designated hepatitis GB virus type C (HGBV-C) and hepatitis G virus (HGV), were identified from patients with non-A-E hepatitis (Simons *et al.* 1995b). The amino acid sequences of the two isolates are 95% homologous, and the genomic organization of this virus places it in the family Flaviviridae (Simons *et al.* 1995a), which includes HCV. The increased risk of HD patients for HCV infection is likely to extend to a recently identified HGV infection which may cause acute and chronic hepatitis. The aim of this study was to assess the prevalence and clinical significance of HGV infection in Korean HD and continuous ambulatory peritoneal dialysis (CAPD) patients.

MATERIALS AND METHODS

Patients

This cross-sectional study was performed in 61 HD patients and 79 CAPD patients at Severance Hospital, Yonsei University in February 1997. The patients were randomly selected from a population of 519 patients with end-stage renal disease undergoing dialysis treatment at our unit. Hospital records for each patient were reviewed for medical history, liver function test, HBV and HCV serology, history of hepatitis and the amount of blood transfusion. A liver function test was performed every month and an episode of hepatitis was defined when alanine aminotransferase (ALT) was greater than 1.5 times the upper value of the normal range.

Methods

All serum samples were collected in February 1997, aliquoted and stored at −70°C. HGV RNA was extracted from 70 *ul* of serum with 200 *ul* Tri-Reagent solution (Molecular Research Center, Cincinnati, OH, USA) and it was converted to complementary DNA (cDNA) with random primer (TaKaRa Biochemicals, Kyoto, Japan) and Avian myeloblastosus virus (AMV) reverse transcriptase (Poscochem, SungNam). Reverse transcribed cDNA was subjected to the first round PCR with primers HG-1 (5'-ggt-cgt-aaa-tcc-cgg-tca-cg-3', 20 mer) and HG-1R (5'-ccc-act-ggt-cct-tgt-caa-ct-3', 20 mer). PCR was performed with TaKaRa Ex Taq (TaKaRa Biochemicals, Kyoto, Japan) for 40 cycles (consisting of denaturation for 15 seconds at 94°C, annealing for 15 seconds at 58°C, and extension for 30 seconds at 72°C). The second round PCR was carried out for 25 cycles (consisting of denaturation for 15 seconds at 94°C, annealing for 15 seconds at 58°C, and extension for 30 seconds at 72°C) with primers HG-2 (5'-ttg-gta-gcc-act-ata-ggt-ggg-tct-3', 24 mer) and HG-2R (5'-att-gaa-ggg-cga-cgt-gga-cc-3', 20 mer). The finding of 188 base pair products on electrophoresis would confirm the presence of HGV-RNA in the test serum.

HBV markers such as HBsAg, anti-HBs and anti-HBc were determined by enzyme-linked immunoassay (Enzygnost, Behring, Macburg, Germany), and anti-HCV was determined by second generation recombinant enzyme immunoassay (Abott Laboratories). The detection of HCV RNA was performed by RT-PCR assay with primers from the 5'-untranslated region of the viral genome. In one patient, HCV RNA was not tested.

Differences in the frequency between groups were analyzed by chi-square test and Fisher's exact test. Group means were compared by student t-test and one-way ANOVA. Mann-Whitney test was used for non-parametric data.

RESULTS

Characteristics of patients

Sixty-one HD patients with a mean age of 49.2± 13.4 years and 79 CAPD patients with a mean age

Table 1. Patient characteristics

	HD	CAPD	p value
No. of patients	61	79	
Age (years)	49.2±13.4	46.7±13.0	NS
Sex (M:F)	43:18	44:35	NS
Duration of dialysis (months)	81.8±65.9	44.5±35.5	$p<0.05$
History of transfusion	38 (62.3%)	39 (49.4%)	NS
Amount of transfusion (pints/case)	7.7±17.3	2.1±3.6	$p<0.05$
History of hepatitis	17 (27.9%)	21 (26.6%)	NS
Mean ALT last 6 months (IU/L)	22.4±37.9	14.0±7.4	NS
HBs Ag (+)	5 (8.2%)	5 (6.3%)	NS
Anti-HCV (+)	13 (21.3%)	3 (3.8%)	$p<0.05$
HCV RNA (+)	10 (16.4%)	1 (1.3%)	$p<0.05$

Values are mean±S.D., NS: not significant

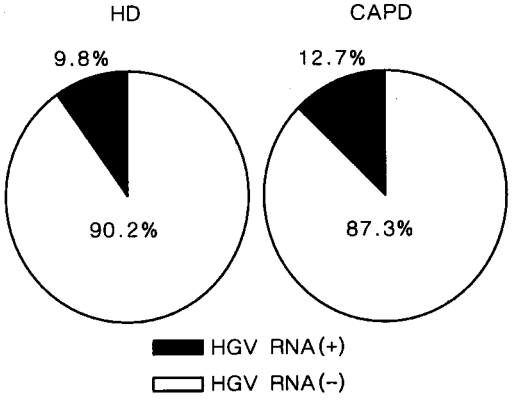

Fig. 1. Prevalence of HGV RNA in HD and CAPD patients

of 46.7±13.0 years were included in our study. Sex, history of transfusion, history of hepatitis, mean levels of ALT in the last 6 months and the detection rate of HBsAg were not significantly different between HD and CAPD patients. However, HD patients had a longer dialysis duration (81.8±65.9 vs. 44.5±35.5 months, $p<0.05$) and received a greater amount of blood transfusion (7.7±17.3 vs. 2.1±3.6 units, $p<0.05$) than CAPD patients. The prevalence of HCV RNA was significantly higher in HD patients compared to that of CAPD patients (16.4% vs. 1.3%, $p<0.05$)(Table 1).

HGV-RNA prevalence

HGV RNA was detected in 6 out of 61 HD patients (9.8%) and in 10 out of 79 CAPD patients (12.7%) without a significant difference (Fig. 1). In both HD and CAPD patients, the presence of HGV-RNA was not related to age, sex, duration of dialysis, history of transfusion, total amount of transfusion, or the presence of HBV and HCV markers. No relationship was observed between HGV RNA positivity and the clinical evidence of hepatitis evaluated by past episodes of hypertransaminasemia and mean levels of ALT during the previous 6 months (Table 2, 3).

Table 2. Clinical data of HD patients according to HGV viremia

	HGV(+) n=6	HGV(−) n=55
Age (years)	53.3±7.6	48.8±13.8
Sex (M:F)	3:3	40:15
Duration of dialysis (months)	125.2±66.3	77.1±64.1
History of transfusion	3 (50%)	35 (63.6%)
Amount of transfusion (pints/case)	1.7±2.7	8.3±18.1
History of hepatitis	2 (33.3%)	10 (18.2%)
Mean ALT last 6 months (IU/L)	18.0±5.7	22.8±39.9
HBs Ag (+)	1 (16.7%)	4 (7.3%)
Anti-HCV (+)	3 (50%)	10 (18.2%)
HCV RNA (+)	2 (33.3%)	8 (14.5%)

Values are mean±S.D.
None of the observed differences was statistically significant.

Clinical significance of HGV infection

The 139 patients who completed the viral study were subdivided into 4 groups according to the presence of either HGV RNA or HCV RNA as shown in Table 4. There were no significant differences in the clinical and biochemical data between patients with isolated HGV infection (n=12) and patients without viremia (n=106). However, HCV RNA positive patients with or without HGV infection (n=11) had a significantly longer duration of dialysis than HCV RNA negative patients (n=128) (data not shown), and the history of hepatitis was more frequently observed in patients with isolated HCV viremia compared to patients without HCV or HGV viremia (55.6% vs. 22.8%, $p<0.05$). Statistical analysis could not be done for the HGV and HCV positive group since the number was too small. The history of transfusion and total amount of transfusion were not different among the other 3 groups. Four of 16 patients with HGV infection were found to be coinfected with HBV or HCV. Mean ALT levels during the previous 6 months for patients with coinfection were similar to those observed in patients with isolated HBV or HCV infection as shown in Tables 4 and 5.

Table 3. Clinical data of CAPD patients according to HGV viremia

	HGV(+) n=10	HGV(−) n=69
Age (years)	47.6±8.4	46.6±13.6
Sex (M:F)	4:6	40:29
Duration of dialysis (months)	47.4±37.3	44.0±35.5
History of transfusion	7 (70%)	32 (46.4%)
Amount of transfusion (pints/case)	5.1±6.2	1.7±2.8
History of hepatitis	5 (50%)	16 (23.2%)
Mean ALT last 6 months (IU/L)	20.1±10.9	13.1±6.4
HBs Ag (+)	1 (10%)	4 (5.8%)
Anti-HCV (+)	1 (10%)	2 (2.9%)
HCV RNA (+)	0	1 (1.4%)

Values are mean±S.D.
None of the observed differences was statistically significant.

Table 5. Clinical data in patients with isolated HBV and HBV/HGV coinfection

	HBV n=8	HBV/HGV n=2
Age (years)	44.9±13.0	56.5±6.4
Sex (M:F)	4:4	1:1
Duration of dialysis (months)	44.1±47.2	8.5±9.2
History of transfusion	6 (75%)	0
Amount of transfusion (pints/case)	3.3±3.5	0
History of hepatitis	3 (37.5%)	0
Mean ALT last 6 months (IU/L)	20.8±18.2	26.0±14.1

Values are mean±S.D.

Table 4. Clinical data of patients according to HGV & HCV RNA status

	HGV(+) HCV(−) n=14	HGV(+) HCV(+) n=2	HGV(−) HCV(+) n=9	HGV(−) HCV(−) n=114
Age (years)	48.4±8.0	59.5±2.1	52.3±7.1	47.3±14.1
Sex (M:F)	5:9	2:0	7:2	73:41
Duration of dialysis (months)	61.6±49.6	181.0±15.6	107.7±51.7[a,b]	55.2±51.4
History of transfusion	9 (64.2%)	1 (50%)	6 (66.7%)	60 (52.6%)
Amount of transfusion (pints/case)	4.3±5.6	0.5±0.7	6.8±7.8	4.5±13.0
History of hepatitis	6 (42.9%)	1 (50%)	5 (55.6%)[b]	26 (22.8%)
Mean ALT last 6 months (IU/L)	19.2±9.8	20.3±2.5	20.0±4.5	17.2±28.5
HBs Ag (+)	2 (14.3%)	0	0	8 (7.2%)

Values are mean±S.D.
[a]: $p<0.05$ vs. HGV(+), HCV(−) group, [b]: $p<0.05$ vs. HGV(−), HCV(−) group

DISCUSSION

A recently discovered HGV is a single-stranded RNA virus that can be categorized as belonging to the Flaviviridae family on the basis of structure (Simons *et al.* 1995a). It is similar in sequence to HGBV-C and may actually be the same virus with a different genotype (Linnen *et al.* 1996; Pilot-Matias *et al.* 1996). Although little is known about transmission, epidemiology and its clinical significance, it has been reported that: HGV may be transmitted via blood products; HGV-related disease is generally mild; HGV and HCV can be transmitted simultaneously and result in coinfection; and HGV infection can persist and result in chronic hepatitis (Linnen *et al.* 1996; Masuko *et al.* 1996). Previous data demonstrated that the risk of HGV infection is increased in patients with a high risk of parenteral exposure, such as hemophiliacs, polytransfused patients with chronic anemia, intravenous drug abusers and HD patients (Linnen *et al.* 1996). Masuko *et al.* and Sampietro *et al.* reported 3.1% and 19% prevalence of HGV infection in HD patients, respectively, however there are little data on HGV prevalence in CAPD patients (Masuko *et al.* 1996; Sampietro *et al.* 1997). Recently, Fabrizi et al. reported 6% prevalence of HGV infection in HD patients and 17% in CAPD patients (Fabrizi *et al.* 1997). However, it is difficult to compare the prevalence between HD and CAPD patients and to demonstrate the clinical significance or risk factors of HGV infection in CAPD patients since too few patients (n=6) were included in the CAPD group.

It is known that patients on CAPD require neither extracorporeal circulation nor manipulation of blood and fewer transfusions are required than for those on HD. Therefore CAPD may reduce the chance of parenterally-transmitted infection. In the case of HCV infection, the reported prevalence of HCV infection in CAPD patients has ranged from 5.0~15.4% (Huang *et al.* 1992; Rafael *et al.* 1992), which is significantly lower compared to 7.4~54.0% in HD patients (Gilli *et al.* 1990; Jeffers *et al.* 1990; Zeldis *et al.* 1990).

Transfusion has been thought to be the major route of transmission for HCV, however, the introduction of the screening of blood products for HCV makes acquisition of this virus by transfusion very unlikely (Donahue *et al.* 1992). Several reports suggested that nosocomial transmission within the hemodialysis units appeared to represent the main mechanism of HCV transmission (Sampietro *et al.* 1995; Stuyver *et al.* 1996). It seemed possible that similar mechanisms of transmission could operate in the acquisition of HGV. Transfusion was thought to be the main mechanism of HGV transmission, and several reports suggested that there might be a common route of transmission of HGV and HCV (Masuko *et al.* 1996; Tsuda *et al.* 1996).

Fabrizi *et al.* reported that the frequency of anti-HCV antibody was significantly higher in HGV-positive than in HGV-negative patients on chronic HD treatment, and the rate of coinfection with HGV and HCV was 87.5%. In our study, however, the prevalence of HGV infection was not different between HD and CAPD patients, while that of HCV infection was significantly higher in HD patients compared to CAPD patients. Furthermore, coinfection with HGV and HCV was observed in only two patients. It seemed unlikely that common routes of transmission operate in the acquisition of HCV and HGV. The presence of HGV RNA was not related to transfusion history, total amount of transfusion or duration of dialysis. In view of these results, it seemed possible that factors unrelated to blood transfusion or nosocomial infection such as direct patient-patient transmission are involved in HGV transmission in dialysis patients.

Clinical features of patients with isolated HGV infection were indistinguishable from those of non-viremic patients. Although six patients with isolated HGV infection had episodes of hypertransaminasemia, the causative role of HGV was not clear since the frequency of hypertransaminasemia was not significantly different from that of non-viremic patients, and neither the drug history nor the presence of hepatic congestion was evaluated in this study. To further clarify the role of HGV in the pathogenesis of liver disease in dialysis patients, more studies are necessary.

In conclusion, we have shown that the prevalence of HGV infection was not different according to the dialysis modality, and that there may be routes of transmission other than transfusion or contamination during the HD procedure. Further studies are needed

to determine the transmission mechanism, natural history and clinical significance of HGV infection.

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