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World J Gastroenterol 2003;9(4):741-744
World Journal of Gastroenterology
 ISSN 1007-9327

• VIRAL HEPATITIS •

TT virus and hepatitis G virus infections in Korean blood donors and patients with chronic liver disease

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Received: 2002-07-02 **Accepted:** 2002-07-27

Abstract

AIM: To determine the prevalences of TTV and HGV infections among blood donors and patients with chronic liver disease in Korea, to investigate the association of TTV and HGV infections with blood transfusion, and to assess the correlation between TTV and HGV viremia and hepatic damage.

METHODS: A total of 391 serum samples were examined in this study. Samples were obtained from healthy blood donors (n=110), hepatitis B surface antigen (HBsAg)-positive donors (n=112), anti-hepatitis C virus (anti-HCV)-positive donors (n=69), patients with type B chronic liver disease (n=81), and patients with type C chronic liver disease (n=19). TTV DNA was detected using the hemi-nested PCR. HGV RNA was tested using RT-PCR. A history of blood transfusion and serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were also determined.

RESULTS: TTV DNA was detected in 8.2 % of healthy blood donors, 16.1 % of HBsAg-positive donors, 20.3 % of anti-HCV-positive donors, 21.0 % of patients with type B chronic liver disease, and 21.1 % of patients with type C chronic liver disease. HGV RNA was detected in 1.8 % of healthy blood donors, 1.8 % of HBsAg-positive donors, 17.4 % of anti-HCV-positive donors, 13.6 % of patients with type B chronic liver disease, and 10.5 % of patients with type C chronic liver disease. The prevalence of TTV and HGV infections in HBV- or HCV-positive donors and patients was significantly higher than in healthy blood donors (P<0.05), except for the detection rate of HGV in HBsAg-positive donors which was the same as for healthy donors. There was a history of transfusion in 66.7 % of TTV DNA-positive patients and 76.9 % of HGV RNA-positive patients (P<0.05). No significant increase in serum ALT and AST was detected in the TTV- or HGV-positive donors and patients.

CONCLUSION: TTV and HGV infections are more frequently found in donors and patients infected with HBV or HCV than in healthy blood donors. However, there is no significant association between TTV or HGV infections and liver injury.

Jeon MJ, Shin JH, Suh SP, Lim YC, Ryang DW. TT virus and hepatitis G virus infections in Korean blood donors and patients with chronic liver disease. *World J Gastroenterol* 2003; 9(4): 741-744
http://www.wjgnet.com/1007-9327/9/741.htm

INTRODUCTION

A novel human DNA virus, TT virus (TTV), was first discovered in the sera of three Japanese patients with post-transfusion hepatitis in 1997[1]. TTV is a non-enveloped, single-stranded virus related to the Circoviridae family[2, 3]. Hepatitis G virus (HGV) is an enveloped RNA virus member of the Flaviviridae family[4]. TTV and HGV infections in healthy blood donors as well as in patients with liver disease have been recently reported in many areas of the world[3-22]. However, the role of these viruses in disease remains uncertain. Information regarding TTV and HGV infections in the Korean population is limited. Hepatitis B virus (HBV) and hepatitis C virus (HCV) are the viral agents most readily implicated in causing a liver disease in Korea[23, 24]. The aims of this study were to determine the prevalence of TTV and HGV in Korean blood donors and patients with type B or C chronic liver disease, to investigate the association between blood transfusion and TTV and HGV infections, and to assess the correlation between TTV and HGV viremia and hepatic damage.

MATERIALS AND METHODS

Materials

A total of 291 blood samples, derived from 110 healthy donors, 112 hepatitis B surface antigen (HBsAg)-positive donors, and 69 anti-hepatitis C virus antibody (anti-HCV Ab)-positive donors, were collected at the Kwangju-Chonnam Red Cross Blood Center. A total of 100 blood samples were obtained from 81 patients with type B chronic liver disease (46 chronic hepatitis, 15 liver cirrhosis, and 20 hepatocellular carcinoma) and 19 patients with type C chronic liver disease (10 chronic hepatitis and 9 hepatocellular carcinoma) at the Chonnam National University Hospital.

Serological and chemical studies

Blood samples were centrifuged and stored at -70 ℃ within hours of collection. HBsAg and anti-HCV Ab were tested with enzyme immunoassay (Axsym, Abbott Laboratories, USA). Immunoblot assay (ProfiBlot IIN, SLT Lab Instruments, Austria) was used to confirm anti-HCV. Serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were also measured using an autoanalyzer (Hitachi 747, Hitachi, Japan).

DNA extraction and TTV PCR

DNA was extracted from 250 μL of sera using a kit (DNAzol, Molecular Research Center, USA) according to the manufacturer' s guidelines. Nucleic acids were dissolved in 50 μL of NaOH (8 mM). A 7 μL volume was then removed and used for amplification of TTV DNA. The PCR reaction was performed in a volume of 50 μL containing 10 mM Tris-HCl

(pH 8.3), 50 mM KCl, 1.5 mM MgCl2, 200 μM dNTPs, 0.5 μM of NG059 (5'-ACAGACAGAGGAGAAGGCAACATG-3') as the sense primer, 0.5 μM of NG063 (5'-CTGGCATTTTACCATTTCCAAAGTT-3') as the antisense primer, and 2 U of Taq polymerase to amplify a 286-bp product. The second-round PCR was done under identical conditions, except that the template was 5 μL product from the first-round PCR, and the sense primer was 0.5 μM of NG061 (5'-GGCAACATGTTATG GATAGACTGG-3'). The size of the second-round PCR product was 271 bp. Each PCR was performed in a programmable thermal cycler (GeneAmp PCR System 9 600, Perkin-Elmer Cetus, USA). The PCR program consisted of 35 cycles of denaturation for 15 seconds at 95 ℃, annealing for 30 seconds at 58 ℃, and extension for 30 seconds at 72 ℃, followed by a final extension for 10 minutes at 72 ℃. The amplification products were separated by 2 % agarose gel electrophoresis, stained with ethidium bromide, and photographed under UV light.

Nucleotide sequencing

To ascertain the specificity of the PCR products, DNA sequences of the amplified product were determined. The PCR product was purified with a PCR purification kit (Boehringer Mannheim, Mannheim, Germany). Nucleotide sequences of the amplicon were directly determined using an AccuPower DNA sequencing kit (Bioneer, Korea). The sequence homology between the PCR products and published TTV DNA was examined. Two randomly selected PCR products were sequenced, both of which were positive for TTV DNA. Sequence comparison with databases confirmed the specific amplification of TTV genomic DNA.

RNA extraction and HGV RT-PCR

To detect HGV RNA in a sample, nucleic acids were isolated from 250 μL of serum using Trizol LS Reagent (GIBCO, USA) and a reverse-transcription PCR (RT-PCR) was performed. A total of 35 cycles of PCR (94 ℃ for 30 seconds, 50 ℃ for 30 seconds, and 72 ℃ for 60 seconds for each cycle) were completed in a programmable thermal cycler (GeneAmp PCR System 9 600, Perkin-Elmer Cetus, USA). The size of the PCR product was 234 bp. The amplification products were separated by 2 % agarose gel electrophoresis, stained with ethidium bromide, and photographed under UV light.

Transfusion history

To examine the association of TTV and HGV infections with blood transfusion, previous transfusion history was investigated in both patients with chronic liver disease and blood donors, by examining their medical records or via telephone interviews.

Statistical analysis

Statistical analysis was conducted using the Chi-square test and analysis of variance (ANOVA). Statistical significance was set when $P<0.05$.

RESULTS

Prevalences of TTV DNA and HGV RNA

The prevalences of TTV and HGV infections in blood donors and patients with chronic liver disease were shown in Table 1. TTV DNA was detected in 8.2 % (9/110) of healthy blood donors, 16.1 % (18/112) of HBsAg-positive donors, 20.3 % (14/69) of anti-HCV-positive donors, 21.0 % (17/81) of patients with type B chronic liver disease, and 21.1 % (4/19) of patients with type C chronic liver disease. The TTV prevalence was significantly higher both in HBsAg-positive or anti-HCV-positive donors and in patients with chronic liver disease than in healthy blood donors ($P<0.05$). HGV RNA was detected in 1.8 % (2/110) of healthy blood donors, 1.8 % (2/112) of HBsAg-positive donors, 17.4 % (12/69) of anti-HCV-positive donors, 13.6 % (11/81) of patients with type B chronic liver disease, and 10.5 % (2/19) of patients with type C chronic liver disease. The HGV prevalence was significantly lower in healthy blood donors or HBsAg-positive donors than in patients with chronic liver disease and anti-HCV-positive donors ($P<0.05$). The detection rates for TTV or HGV were not significantly different between the three types of chronic liver disease. The TTV prevalence was about 4.6 times higher than the HGV prevalence in healthy blood donors. Co-infection of TTV and HGV was also observed in ten cases of HBV- or HCV-infected patients and donors.

Table 1 The prevalence of TTV and HGV infections in blood donors and patients with chronic liver disease from Korea

Subject	*n*	TTV (%)	HGV (%)	TTV & HGV (%)
Healthy blood donors	110	9 (8.2)	2 (1.8)	0 (0.0)
HBsAg(+) donors	112	18 (16.1)[a]	2 (1.8)	0 (0.0)
Anti-HCV(+) donors	69	14 (20.3)[a]	12 (17.4)[b]	3 (4.3)
Patients with type B chronic liver disease	81	17 (21.0)[a]	11 (13.6)[b]	6 (7.4)
Chronic hepatitis	6	10(21.7)	5 (10.9)	3 (6.5)
Liver cirrhosis	15	3 (20.0)	3 (20.0)	2 (13.3)
Hepatocellular carcinoma	20	4(20.0)	3 (20.0)	1 (5.0)
Patients with type C chronic liver disease	19	4 (21.1)[a]	2 (10.5)[b]	1 (5.3)
Chronic hepatitis	10	2 (20.0)	1 (10.0)	0 (0.0)
Hepatocellular carcinoma	9	2 (22.2)	1 (11.1)	1 (11.1)
Total	391	62 (15.9)	29 (7.4)	10 (2.6)

[a]$P<0.05$ *vs* healthy blood donors, [b]$P<0.05$ *vs* healthy blood donors or HBsAg-positive donors.

Association of TTV and HGV infections with blood transfusion

To investigate the association of TTV and HGV infections with blood transfusion, the medical records for the previous transfusion history were reviewed in patients with chronic liver disease. The rate of transfusion history was higher in TTV- or HGV-positive patients than in TTV- or HGV-negative patients ($P<0.05$) (Table 2).

Table 2 Transfusion history according to the detection of TTV and HGV in patients with chronic liver disease

Group		*n*	Transfusion history	
			Yes (%)	No (%)
TTV	Positive	21	14 (66.7)[a]	7 (33.3)
	Negative	77	33 (42.9)	44 (57.1)
HGV	Positive	13	10 (76.9)	3 (23.1)
	Negative	85	37 (43.5)	48 (56.5)
Total		98	47 (48.0)	51 (52.0)

[a]$P<0.05$ *vs* TTV-or HGV-negative patients.

Correlation between TTV or HGV viremia and hepatic damage

To evaluate the correlation between TTV or HGV infections and hepatic damage, the serum levels of ALT and AST were measured in all subjects (data not shown). No significant increase in serum ALT and AST was observed in TTV- or HGV-positive blood donors, compared with TTV- or HGV-negative donors. In addition, TTV or HGV co-infection did

not elicit any further significant increase in ALT and AST levels in patients with chronic liver disease. Furthermore, no increase in ALT and AST was observed in HBV- or HCV-positive patients or donors infected with both TTV and HGV. These observations suggested that there is no significant association between TTV or HGV infections and hepatic injury.

DISCUSSION

TTV was detected in 8.2 % of healthy blood donors from Korea. The prevalence of TTV infection among blood donors in other countries is 1.9 % in the United Kingdom[5], 3.2 % in Germany[6], 7.5-10 % in the United States[7, 8], 12 % in Japan[3], 29.4 % in Egypt[9], 36 % in Thailand[10], and 62 % in Brazil[11]. These differences in prevalence between countries could be due to the different geographical distribution of TTV infections, and the heterogeneity and variability of TTV isolates[3, 5]. Variation could also arise due to different experimental methods to determine TTV infection, such as the primers used, and the sensitivity of the PCR methods employed[8, 25]. The primer used in our study was identical to that used in the aforementioned countries, suggesting that the discrepancies of TTV prevalence between countries were not due to variation in the primer used. The detection rate of HGV RNA in blood donors from many other countries ranged from 0.5 to 7.4 %[19-22]. In healthy Korean blood donors, the detection rate of HGV was 1.8 %, and the prevalence of TTV was higher (about 4.6 times) than that of HGV.

We observed TTV and HGV co-infection in subjects with HBV or HCV findings that had also been reported elsewhere[12-18]. The co-infection rate of TTV in HBV- and HCV-infected subjects was similar, a finding also reported by others[10, 12]. However, there was some difference in the co-infection rate of HGV between HBV and HCV. The prevalence of HGV in HBsAg-positive donors was lower than in patients with chronic liver disease and also anti-HCV-positive donors. Giulivi *et al*. have reported similar findings[26]. The discrepancy in the co-infection rate of HGV between HBV- and HCV-positive donors may have clinical significance. With the exception of HGV co-infection in HBV-positive donors, both HBV- and HCV-infected subjects had a higher detection rate of TTV or HGV than healthy donors. This suggested that HBV- or HCV-infected subjects had a greater exposure to a risk factor for viral infections. These viruses may also share a common route of transmission.

A total of 66.7 % of TTV DNA-positive patients and 76.9 % of HGV RNA-positive patients had a history of blood transfusion. This suggests the possibility of viral transmission via blood transfusion. TTV and HGV are prevalent in the sera of persons with hemophilia, intravenous drug users, and hemodialysis patients[3, 8, 21, 27]. However, our results indicate that TTV and HGV were observed in donors and patients without a transfusion history, which suggests a non-parenteral route of transmission. TTV and HGV have reportedly been detected in urine, feces, and breast milk[6, 28, 29]. Thus, TTV and HGV might be transmitted via several parenteral and non-parenteral routes.

No significant increase in ALT or AST was observed in healthy donors or liver disease patients infected with either TTV or HGV, compared to subjects without these viral infections. Kao *et al*. reported that TTV infection does not affect the disease process of type B or C hepatitis, or the response to interferon treatment[15]. Oguchi *et al*. demonstrated that a TTV carrier state was maintained without hepatitis in hemodialysis patients over a 5-year follow-up period[30]. Alter *et al*. showed that HGV infection was not associated with hepatitis and did not worsen the course of concurrent HCV infection[31]. Considering all these results, TTV or HGV infection seems not to be related to the initiation or potentiation of hepatic damage, which therefore suggests that the routine screening test for TTV and HGV on donated blood is not necessary. At present there also is no country that conducts the screening test for these viral infections.

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Edited by Ma JY and Xu XQ