• ORIGINAL RESEARCH •

High frequencies of HGV and TTV infections in blood donors in Hangzhou

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Abstract

AIM **To determine the frequencies of HGV and TTV infections in blood donors in Hangzhou.**

METHODS **RT-nested PCR for HGV RNA detection and semi-nested PCR for TTV DNA detection in the sera from 203 blood donors, and nucleotide sequence analysis were performed.**

RESULTS **Thirty-two (15.8%) and 30 (14.8%) of the 203 serum samples were positive for HGV RNA and TTV DNA, respectively. And 5 (2.5%) of the 203 serum samples were detectable for both HGV RNA and TTV DNA. Homology of the nucleotide sequences of HGV RT-nested PCR products and TTV semi-nested PCR products from 3 serum samples compared with the reported HGV and TTV sequences was 89.36%, 87.94%, 88.65% and 63.51%, 65.77% and 67.12%, respectively.**

CONCLUSION **The infection rates of HGV and/or TTV in blood donors are relatively high, and to establish HGV and TTV examinations to screen blood donors is needed for transfusion security. The genomic heterogeneity of TTV or HGV is present in the isolates from different areas.**

Subject headings China; DNA virus infections/epidemiology; hepatitis, virus, human/ epidemiology; blood transfusion/ adverse effect; blood donors; hepatitis agents, GB/ isolation & purification

INTRODUCTION

Viral hepatitis is relatively common in China[1-12]. Among them hepatit is G virus (HGV) and hepatitis GB virus (GBV-C) were recently identified as the two isolates of a novel positive-stranded RNA virus belonging to *Flaviviridae* family associated with human non-A-E hepatitis[13,14]. In 1997, a negatively stranded DNA virus, named transfusion transmitted virus (TTV), was isolated from a patient suffering from non-A-G hepatitis. At present TTV was proposed to be a member of a new virus family temporarily named *Circinoviridae*[15,16]. HGV RNA could be detected in patients with non-A-E hepatitis or fulminant hepatic failure (FHF) at relatively high percentages[17-22] and the coinfection of HGV and HCV may accelerate the progression of chronic liver disease[23-25]. However, many investigation data revealed that HGV was able to establish a long-term asymptomatic viremia in non A-E hepatitis patients and only a few of the patients had biochemical evidence of liver damage[26-30]. TTV DNA was frequently found in n on-A-G hepatitis patients with a single elevation of alanine aminotransferase (ALT) and the persistence of TTV infection might be a causative factor of human FHF[31-33]. A high frequency of TTV virus infection among patients with non-B, non-C hepatocellular carcinoma was also reported by Nakagawa et al[34]. However, some literatures reported that clinical implication of TTV infection was insignificant because of minimal role of liver injury in non-A-G hepatitis patients[35-37]. The clinical importance of HGV and TTV infections in human hepatic diseases is still present even though the real pathogenic potentials of the two viruses have remained unanswered. Therefore, the prevalence of HGV and TTV infections in blood donors is a significant subject for investigation.

In the present study, HGV RNA and TTV DNA in the serum samples of 203 blood donors in Hangzhou, eastern China, were detected using RT-nested PCR and semi-nested PCR, respectively. The HGV RNA or TTV DNA positive amplification products from part of the serum samples were cloned and sequenced. The results of this study may help determine the frequencies of HGV and TTV infections in blood donors in the local area and provide the basis for screening blood donors to control transmission of the two viruses.

MATERIALS AND METHODS

Materials

A total of 203 serum samples of healthy blood donors were obtained from four hospitals in Hongzhou, Zhejiang Province of China. The blood donors were confirm ed to have neither clinical signals of hepatitis and nor elevation of ALT by conventional hepatic examinations, and the serum samples were negative for hepatitis A-C viruses by EIA and PCR. The reagents used in reverse transcription (RT) and PCR were purchased from Sangon and the other reagents used in this study from Sigma.

Methods

Isolation of serum RNA and DNA Total RNA in 200 μL of each serum samples was prepared by Trizol method according to the manufacturer's instruction and then dissolved in 50 μL

of DEPC treated water. Total DNA in 200 μL of each serum samples was extracted by phenol-chloroform method[38], and then dissolved in 50 μL TE buffer (pH 8.0).

RT-nested PCR for HGV RNA detection Ten microliters of total RNA preparation was mixed with 10 μL RT master mixture containing 1 μmol·L^{-1} of random hexanucleotide as primer, 2 mol·L^{-1} each of dNTP, 20U M-MuLV-reverse transcrip tase, 20U RNase inhibitor and 4 μL of 5× RT buffer (pH 8.3). The steps of RT were described as follows: at 70 ℃×5 min for denaturation, at 42 ℃×60 min for cDNA synthesis, and at 70 ℃×10 min to stop the reaction.

Primers derived from HGV 5'-NCR were used in the RT-nested PCR[39]. External primers: 5'-ATGACAGGGTTG-GTAGGTCGT AAATC-3' (sense), 5'-CCCCACTGGTC-CTTGTCAACTCGCCG-3' (antisense). Internal primers: 5'-TGGTAGCCACTATAGGTGG GTCTTAA-3' (sense), 5'-ACATTGAAGGGCGACGTGGACCGTAC-3' (antisense). For the first round PCR, 10 μL of RT product was mixed with 90 μL PCR master mixture containing 250 nmol·L^{-1} each of the primers, 2 mol·L^{-1} each of dNTP, 25 mol·L^{-1} MgCl₂, 2.5 U of Taq DNA polymerase and 10 μL of 10×PCR buffer (pH 9.1). For the second round PCR, 5 μL product from the first round PCR was used as template, and the other reaction reagents were the same as that in the first round PCR except for the primers. The parameters for each of the PCR rounds were: 94 ℃ 5 min (×1); 94 ℃ 1 min, 56 ℃ 1 min, 72 ℃ 1.5 min (×35) and 72 ℃ 7 min (×1). The expected size of target fragments amplified from HGV RNA was 193 bp.

Semi-nested PCR for TTV DNA detection The primers used in the semi-nested PCR for TTV DNA detection were the same des cribed by Okamoto *et al*[40]. External primers: 5'-ACAGACAGAGGAGA AGGCAACATG-3' (sense), 5'-CTGGCATTTTACCATTTCCAAAGTT-3' (antisense). Inte rnal primers: 5'-GGCAACATGTTATGATAGACTGG-3' (sense), 5'-CTGGCATTTTACCATT TCCAAAGTT-3' (antisense). Except for specific primers, MgCl₂ concentration (15mol·L-1), total reaction volume (50 μL) and annealing temperature (60 ℃), the compositions and concentrations of other reaction agents and the parameters for semi-nested PCR was the same as that of the RT-nested PCR for HGV detection. The expected size of target fragment s amplified from TTV DNA was 271 bp.

Examination of amplification products The results of amplificat ion reactions were observed on UV light after 20 g·L^{-1} ethidium bromide stained agarose electrophoresis, and 100 bp DNA ladder was used as a size marker to estimate the length of products.

Analysis of nucleotide sequences of amplification products The target DNA fragments from HGV or TTV amplification products by PCR were cloned into plasmid pUCm-T-vector using T-A cloning kit according to the manufa cturer's instruction. The recombinant plasmid was amplified in *E.coli* strain DH 5α and then recovered by Sambrook's method[38]. The nucleotide sequence of inserted fragment was analyzed by Sangon. Homology of the nucleotide sequences was compared with those of reported[13,40].

RESULTS

Positive rates of HGV RNA and TTV DNA in the serum samples
The respective target fagments respectively amplified from HGV RNA and TTV DNA are shown in Figure 1. Thirty-two (15.8%) 30 (14.8%) and 5 (2.5%) of the 203 serum samples were positive for HGV RNA, TTV DNA and both of the two respectively (Table 1).

Table 1 Positive rates of HGV RNA and TTV DNA in the 203 blood donors

Virus	Tested (*n*)	Positive (*n*)	%
HGV	203	32	15.8
TTV	203	30	14.8
HGV and TTV	203	5	2.5

Nucleotide sequence analysis and homology comparison
Homology of the nucleotide sequences of HGV RT-nested PCR products from 3 serum samples compared with the reported HGV sequence[13] was 89.36%, 87.94% and 88.65% respectively (Figure 2). Homology of the nucleotide sequences of TTV semi-nested PCR products from 3 serum samples compared with the reported TTV TA278 genotype-1a sequence[40] was 63.51%, 65.77% and 67.12% (Figure 3). The homology in comparison of these sequences mentioned above did not contain the primer sequences.
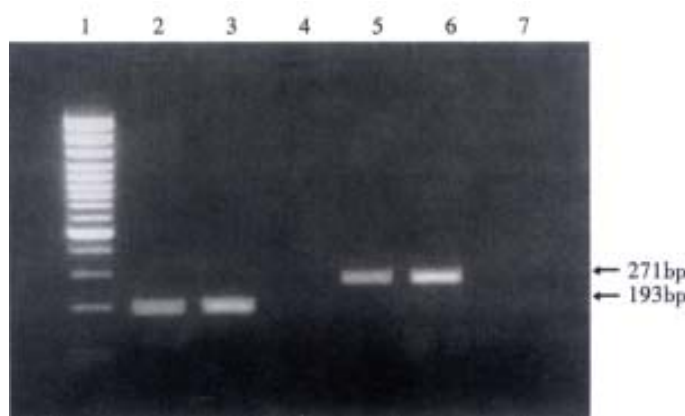

Figure 1 Target amplification fragments from HGV RNA and TTV DNA. (1: marker; 2 and 3: HGV positive serum samples; 4: blank for HGV detection; 5 and 6: TTV positive serum samples; 7: blank for TTV detection)

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1:   1  TCGTAGCCACTATAGGTGGGTCTTAAGAGAAGGTTAAGATTCCTCTTGTGCCTGCGGCGA
2:   1  .............................G.TT...C...G.C.....G.C..T........
3:   1  .............................G.TT...C...G.C.....G.C.....T.....
4:   1  .............................G.TT...C...G.C.....G.C..T..T.....

1:  61  GACCGCGCACGGT CCACAGGTGTTGGCCCTACCGGTGGGAATAAGGGCCCGACGTCAGG
2:  61  A.AA.......................................T....................
3:  61  A.AA.........C.............................T....................
4:  61  ..AA....................A.................T....................

1: 121  CTCGTCGTTAAACCGAGCCCGTTACCCACCTGGGCAAACGACGCCCACGTACGGTCCAGG
2: 121  ...................A.A.......................................
3: 121  ...................A.......G..........A.......................
4: 121  ...................A.....................A....................

1: 180  TCGCCCTTCAATGT
2: 181  ..............
3: 180  ..............
4: 180  ..............
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Figure 2 Homology of the nucleotide sequences from HGV RT-nested PCR products from 3 serum samples as compared with the reported sequence. (1. The reported HGV sequence[13]; 2-4. The sequences of HG V RT-nested PCR products from 3 serum samples. Underlined areas indicate the primer positions.)

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1:   1  GGCAACATGTTATGGATAGACTGGCTAAGCAAAAAAAACATGAACTATGACAAAGTACAA
2:   1  .........................CT...G.TG..TCAGTA...A....GCAG...
3:   1  .........................CT...G.TG..TCAGTA.......GCAG...
4:   1  .........................CT.....GG..TCAGTA.......GCAG...

1:  61  AGTAAATGCTTAATATCAGACCTACCTCTATGGGCAGCACCATATGGATATGTAGAATTT
2:  61  ........TC.....CA.....A...CT.G.....CT...T.........CAC.....AC
3:  61  ..............CA.....A...CT.G.....CT...T.........CAC.....AC
4:  61  ........TC.....CA.........CT.G.....CT............CAC.....AC

1: 121  TGTGCAAAAAGTACAGGAGACCAAAACATACAGATGAATGCCAGGCTACTAATAAGAAGT
2: 121  ..CAGC...GTA.........AC.......G.ACAC...TGT..ATGTG....T.....C
3: 121  ..CAGC...GTA.........AC.........ACAC...TGT..ATGTG....T.....C
4: 121  ..CAGC...GTA.........AC.......G..CAT...TGT..ATGTG....T......

1: 181  CCCTTTACAGACCCACAACTACTAGTACACACAGACCCCACAAAAGGCTTTGTTCCTTAC
2: 181  ....A.....TA..T..G.......AC....ACA..AG..TT.GG..A.AC..A..C...
3: 181  ....A.....TA..T..G.......AC....ACA..AG..TT.GG..A.AC.....C...
4: 181  ..........TA..T..G.......AC....ACA..AG..TT.GG...A.AC.....G...

1: 241  TCTTTAAACTTTGGAAATGGTAAAATGCCAG
2: 241  AGCA...........................
3: 241  AGCA...........................
4: 241  AGCA...........................
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Figure 3 Homology of the nucleotide sequences from T TV semi-nested PCR products from 3 serum samples as compared with the reported sequence.
1. The reported TTV sequence[40]; 2-4. The sequences of TTV semi-nested PCR products from 3 serum samples. Underlined areas indicate the primer positions.

DISCUSSION

Viral hepatitis is a common infectious disease in the world and causes a serious healthy problem. Although hepatitis viruses A-E have been demonstrated to be responsible for human hepatitis A-E, approximately 20% of acute and 15% of chronic hepatitis were associated with unknown aetiology[41]. HGV and TTV were recently identified as the transfusion-transmitted viruses and the causative agents of human non-A-E hepatitis[13-15]. However, many investigation data revealed that the patients infected with HGV or TTV were usually asymp tomatic and only a few of them showed mild liver injury[42-44]. Therefore, a wide variety of questions about the potential pathogenicity of HGV and TTV infection remain unanswered[31,45-47].

Since HGV and TTV are generally transmitted by transfusion, high infection frequencies of the two viruses in blood recipients and in hemodialyzed patients have been demonstrated[31,43,48-50]. Many investigations demonstrated that HGV RNA was detectable in 1.3%-10.6% of blood donors in different areas abroad[51-55]. Blood donors were also frequently infected with TTV but the infection rates abroad were usually lower than 5%[56,57]. In China, HGV and TTV infection rates in blood donors were reported to be approximately 8% and 15%, respectively[46,58]. In the present study, HGV infection rat e in the 203 blood donors was as high as 15.8%, which seems obviously higher than the reported HGV infection rates in the blood donors from other areas of China and abroad. Such high HGV infection rate in the blood donors in Hangzhou is probably due to the geographic difference. In this study, TTV viremia was found in 14.8% of the same 203 blood donors, which is higher than that of the reported abroad but similar to the reported in Chinese blood donors from other areas. In addition, 5 (2.5%) serum samples from the 203 blood donors were positive for both HGV RNA and TTV DNA, indicating the existence of coinfection of the two viruses. However, we can not exactly evaluate the significance of the co-infection because of being unable to get the detailed information of the five co-infection blood donors.

None of the blood donors tested in this study showed clinical symptoms and laboratory markers for hepatitis, which suggested that most of blood donors infected with HGV and/or TTV are usually latent. These asymptomatic blood donors carrying HGV and/or TTV may be more risky and important for the source of infection. Since HGV and TTV at least cause mild hepatitis in human[15,41] and high frequency of HGV and/or TTV infections in blood donors, it is necessary to establish HGV and TTV examination items to screenblood donors for transfusion security.

The nucleotide sequences of HGV RT-nested PCR products from 3 serum samples¡¡are highly homologous (87.94%-89.36%) to the HGV sequence reported by Linnen et al[13]. This result of sequence analysis indicates that the RT-nested PCR used in this study is reliable for HGV RNA detection. To analyze the details of the nucleotide sequencing data, it seems to show an HGV genotype different from the literature[59-62]. Lower homology (63.51%-67.12%) of the nucleotide sequences of TTV semi-nested PCR products from 3 serum samples compared with the reported TTV sequence[40] reveals the genomic heterogeneity of TTV in the isolates from different areas, and this founding accords with the conclusions of previously published reports[37,40].

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