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The prevalence of transfusion transmitted virus infection in blood donors

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INTRODUCTION

A newly discovered DNA virus, transfusion transmitted virus (TTV), was reported as a cause of post-transfusion hepatitis of unknown etiology in Japan[1]. In order to investigate TTV prevalence in southern China, a study was carried out among blood donors, patients with liver diseases and hemodialysis to deter mine the epidemiological charateristics.

MATERIALS AND METHODS

Samples

Sera or plasma samples (471) were obtained from volunteer blood donors from Shen zhen Baoan Blood Center and commercial blood donors from Dongguan Blood Center. Sera samples (117) from patients with liver disease and hemodialysis were collected from Shenzhen Baoan People's Hospital. All the sera or plasma samples were stored at -70℃ for detection.

Reagents

Hepatitis B surface antigen (HBsAg), antibodies to hepatitis C virus (HCV), antibodies to human immuno-deficiency virus (HIV) EIA kits were purchased from Abbott Laboratories (Abbott Park, IL). Syphlis TPHA kits were purchased from Randox Laboratories Ltd. IgM antibodies to hepatitis A virus, antibodies to hepatitis E virus ELISA reagent kits, HBV, HCV and HGV polymerase chain reaction (PCR) reag ent kits were purchased from the Institute of Hepatology of Beijing Medical Univ ersity.

Laboratory tests

The serum specimens from blood donors were tested for ananine aminotransferase (ALT) levels (less than or equal to 40 IU/L), HBsAg, antibodies to HCV, antibodies to HIV and syphlis TPHA. The patients with liver disease were further detected for markers of HAV, HBV, HCV, HEV and HGV infection.

Detection of TTV DNA by nPCR[2]

Nucleic acid was extracted from 100 μL serum with AcuPure DNA/ RNA extraction kit (Biotronics Tech. Corp.) following the -manufacturer's-recommended protocol. According to the sequence of TTV TA278 strain (GenBank acc esion number AB008394) two pair nested primers were designed for nested polymera se chain reaction (nPCR). The first-round PCR was performed with P1 (sense: 5'-CCAGGAGCATATACAGAC-3') and P2 (anti-sense: 5'-TACTTCTTGCTGGTGAAAT-3') for 30 cycles (pre-denatu ring for 180s at 94 ℃, denaturing for 40s at 94 ℃, annealing for 40s at 55 ℃, extension for 40s at 72 ℃). The second-round PCR was carried out with P3 (sense: 5'-CAGACAGAGGAG-AAGGCAAC-3') and P4 (anti-sense: 5'-ACAGGCACATTACTACTACC- 3') for 30 cycles for amplification of 309bp product. The second-round PCR was done in the same manner as the first-round. Amplified products were separated in 2% agorase gel electrophoresis, stained with ethidium bromide and observed under ultraviolet light.

Cloning and sequencing of TTV genome

The purified products of PCR were directly ligated into pBluescript-T vector (Invitrogen Company), and with recombinant plasmid obtained by introduction into *E. coli* XL1-blue, sequences were determined for both strands by the sanger dideoxy chain termination methods, and with sequencer (model ABI373; App lied Biosystems, Foster City, CA).

Data analysis

The sequences of TTV genome were analyzed by the computer program CLUSTALW version 1.1 and the computer program GOLDKEY version 1.1.

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Statistical analysis was made using statistical program SYSTAT version 3.0.

RESULTS

The results of TTV DNA determined by nPCR in blood donors

Four hundred and seventy-one sera samples from volunteer blood donors and commercial blood donors were all negative for markers of HBsAg, anti-HCV, anti-HIV and TPHA. Fifty-eight of 471 sera samples had an elavated ALT (mean 89 IU/ L±45 IU/L) and with no markers of hepatitis A-G virus infection. The results of TTV DNA detected by nPCR from blood donors are shown in Figure 1 and Table 1. Among volunteer blood donors with normal transaminase levels, 30 (14.7%) of 204 were positive for TTV DNA. In contrast, 48 (23.0%) of 209 commercial blood donors with normal transaminase levels, and 18 (31.0%) of 58 blood donors with elevated transaminase levels were positive for TTV DNA. The prevalence of TTV in blood donors with elevated transaminase levels was significantly higher than in volunteer blood donors ($P<0.01$) and commercial blood donors wit h normal transaminase levels ($P<0.05$). There was no significant differ ence between the commercial blood donors with normal transaminase levels and the blood donors with elevated transaminase levels.

Table 1 The results of TTV DNA detected by PCR in sera of blood donors

Blood donors	No. of sample	TTV DNA (+)	
		n	%
Commercial blood donors with normal ALT levels	209	48	23.1
Volunteer blood donors with normal ALT levels	204	30	14.4
Blood donors with elevated ALT levels	58	18	31.0

The results of TTV DNA detected by PCR in patients with liver diseases and hemodialysis

One hundred and five serum specimens from patients with liver diseases were detected for TTV DNA by nPCR. The results are shown in Table 2. Among 36 non-A to E hepatitis patients, 15 (41.6%) were PCR positive for TTV DNA. One of 13 (7.7%) patients with hepatitis A virus infection was positive for TTV DNA, 3 (23.1%) of 13 patients with hepatitis B virus infection and 8 (18.6%) of 43 patients with hepatitis C virus infection were positive for TTV DNA. The prevalence of TTV in non-A to E hepatitis patients was significantly higher than in patients with HAV or HBV and HCV infection. Among 12 cases of hemodialysis, 5 (41.7%) were PCR positive for TTV DNA.

The sequencing results of TTV partial genome from blood donors

The anticipated DNA fragments amplified by PCR from 5 blood donors were cloned and sequenced for both strands. The similarity of nucleotide sequences of partial gene within TTV ORF1 region among 5 isolates from blood donors ranged from 98.4% to 99.4%. There was a 97% nucleotide identity among AB008394 (from Japan), TTVCHIN1 (from northern China) and our 5 isolates from blood donors (Figure 2 and Table 3).

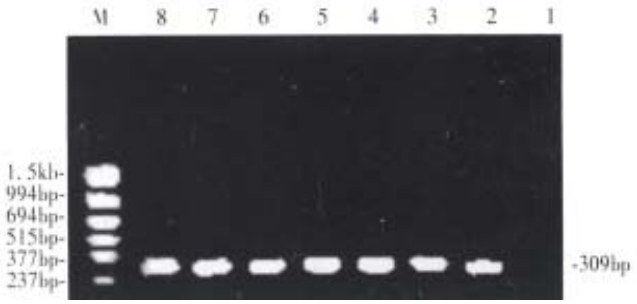

Figure 1 Agorase electrophoresis of TTV DNA PCR products.M: marker; 1: negative control; 2-8: positive

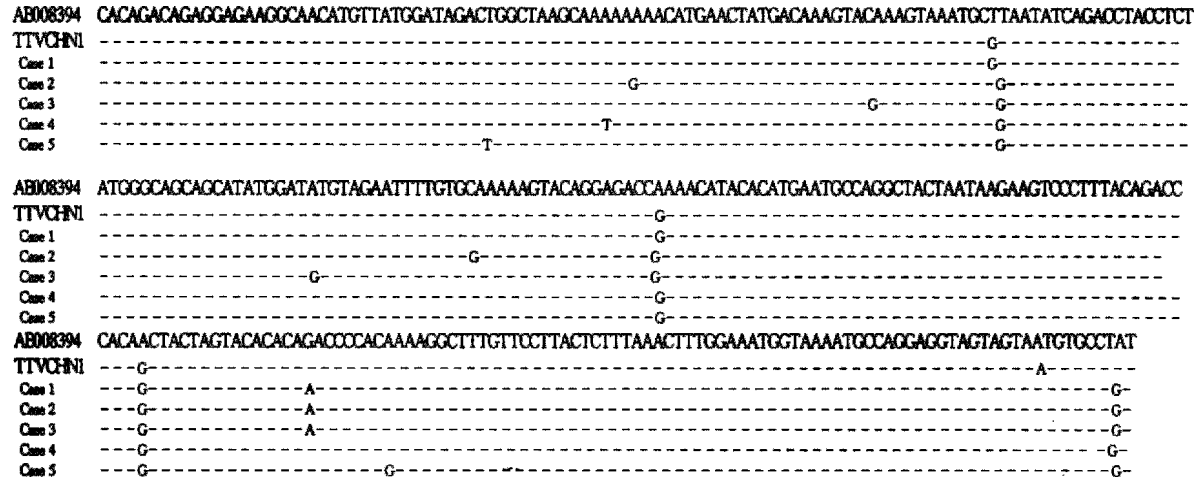

Figure 2 Comparison of isolates from blood donors (cases 1-5) with corresponding sequences from Japan (AB008394) and China (TTVCHN1).

Table 2 The results of TTV DNA detected by PCR in sera of the patients with liver diseases and hemodialysis

Patients	Cases tested	TTV DNA (+)	
		n	%
Non-A to E hepatitis patients	36	15	41.6
Hepatitis A patients	13	1	7.7
Hepatitis B patients	13	3	23.1
Hepatitis C patients	43	8	18.6
Hemodialysis	12	5	41.7

Table 3 Homologies of nucleotide sequences among isolates from blood donors of Japan and China (%)

TTV isolates	AB008394	TTVCHN1	Case 1	Case 2	Case 3	Case 4
TTVCHN1	98.7					
Case 1	98.4	99.7				
Case 2	97.7	99.1	99.4			
Case 3	97.7	99.1	99.4	98.7		
Case 4	98.4	99.7	99.4	98.7	98.7	
Case 5	98.1	99.4	99.1	98.4	98.4	99.1

DISCUSSION

Transfusion transmitted virus (TTV) is a newly discovered virus associated with the patients with post-transfusion hepatitis of unknown etiology in Japan. The preliminary studies showed that TTV was a single-stranded DNA virus, and the nucleotide sequence of TTV DNA genome was composed of approximately 3.7 kp, including two open reading frame (ORF) which encodes for 770 amino acids (aa) and 202 aa, respectively[3]. TTV resembles to some known animal single-stranded DNA viruses, such as chicken anemia virus and human parvovirus B19. Some studies reported that TTV was associated with post-transfusion and acute and chronic he patitis of unknown etiology. So far, no reliable serologic assay for antibodies against TTV infection has been developed. Thus, PCR was utilized to determine the prevalence of TTV infection in different populations.

Based on the conserved nucleotide sequence of TTV ORF1 gene, a nested-PCR for TTV DNA was established in our study. The specificity of amplified targets was documented by sequencing the PCR products selected from 5 blood donors positive for TTV DNA (Figure 2). The epidemiological investigation indicated that there was a high prevalence of TTV infection in volunteer blood donors and commercial blood donors. Simmonds *et al*[4] reported that TTV viraemia was detected in 19 (1.9%) of 1000 non-remunerated regular blood donors from Scotland, 10% from England. Fourty-four to 56% factor VIII and IX had a TTV contamination. In this study, nearly 15%-31% of blood donors from southern China were TTV DNA positive. Hemodialysis patients were considered at "high-risk" for exposure to parenterally transmitted viruses, such as HBV and HCV. TTV DNA positive rate was 41.7 % in our data. The results suggested that TTV can be parenterally transmitted to recipients of blood or blood products.

Fifty-eight blood donors with elevated serum ALT levels were tested without markers of hepatitis A to G viruses. The positive rate of TTV DNA was 31%, which was higher than in volunteer blood donors with normal ALT levels (14-7%). The results indicated that some blood donors with abnormal ALT was associated with TTV infection.

The pathogenicity of TTV is unclear. The clinical and epidemiological studies showed that the TTV infection was found among patients with fulminant hepatitis, acute and chronic liver disease of unknown etiology[3,5]. In our study, the prevalence of TTV was higher in patients with non-A to G hepatitis (41.6%) than in patients with hepatitis A to C virus infection, indicating that TTV may be responsible for some cases of non-A to E hepatitis. Recent studies demonstrated that TTV may cause epidemic outbreak of hepatitis of unknown etiology by fecal-to-mouth way[6]. The high prevalence of active TTV infection (14.7%) in the general population was found in our study, suggesting that TTV, similar to HBV, may have "symptom-free carriers". Further studies are urgently needed to determine the pathogenicity of TTV and the significance of "symptom-free c arriers".

We amplified and sequenced the ORF1 partial gene of TTV genome from 5 blood donors and compared these sequences with those of isolates from Japan and northern China. Our preliminary results showed that there are less than 2% nucleotide divergence among 5 isolates from blood donors. Compared with the isolates from Japan and northern China, the similarities of nucleotide sequences were above 97%. The data suggest that the ORF1 region of TTV genome is conserved in different geographic areas.

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