
Transfusion-Transmitted Virus (TTV) : Positive Rates in Healthy Individuals and Cases with Hepatitis C Antibody

Abstract

Transfusion-transmitted virus (TTV) was first detected and reported by Nishizawa in 1997. Three years later, with more reports coming from all corners of the world, TTV was found to be similar to HGV. The virus exists extensively in human body, and is transmitted primarily through body fluid, though the likelihood of transmission via mouth should not be overlooked. TTV infection is not directly associated with the pathogenic agents of hepatitis. Prevalence of TTV varies with different geographic regions and studies, ranging from 10% to 80-90%. Several studies have been reported from Taiwan. The purpose of the present study was to verify whether the positive rates in Taiwan were different from those reported elsewhere. Both polymerase chain reaction and gene sequencing methods were applied to analyze the infections of TTV-DNA in three study groups: (1) 300 healthy individuals (ALT normal, average age 25.8 years), (2) 53 anti-HCV positive, ALT normal cases

(36 males and 17 females, average age 34.5 years), and (3) 300 anti-HCV positive, ALT abnormal cases (148 males and 152 females, average age 36.4 years). TTV-DNA was measured by polymerase chain reaction, and their genotypes were analyzed by gene sequencing method. TTV-DNA positive rates were 9% and 31% ($p < 0.05$) respectively in the anti-C negative and positive groups, indicating some association between TTV-DNA infection and hepatitis C virus infection. Whether ALT was normal or not, it was not associated with the TTV-DNA positive rate. The main genotype of TTV-DNA in the cases studied was type-1 (accounting for about 73%); and the subtypes were genotype IA (53%) and genotype IB (47%). The present study had also detected four thus far not reported subtypes. The TTV-DNA positive rates in the healthy individuals and anti-HCV positive cases in the present study were somewhat different from rates reported elsewhere in the world and in Taiwan, indicating, as commonly understood, that TTV infection rates differed from place to place and in different groups. Whether the infection was associated with hepatitis C virus infection remained to be studied further. The detection of four new genotypes showed the diversity of TTV genotypes and subtypes.

Introduction

As commonly understood, viruses that can be transmitted via blood transfusion are primarily hepatitis B virus (HBV), and hepatitis C virus (HCV). In 1995, hepatitis G virus and, in 1997, TTV were found. TTV can often be detected in the blood of patients of abnormal liver functions. Current studies suggest that TTV infection is a relatively common virus infection throughout the world in different places and different racial groups^(1,2,3,4,19,20).

TTV is a kind of DNA virus. The virus contains some single ply genes, with

at least 3,739 bases. The virus is 30-50 nm in size, and is non-enveloped. Some features of the virus are similar to those of Parvoviruses, of lower sedimentation density index (1.26g/cm), for instance. The virus has gone through significant mutations, and is now grouped under the *Circinoviridae* virus family. It has two different genotypes. They are different by about 30% in DNA sequences^(5,6).

Since TTV is often detected in blood donors who have used coagulant factor, and also in serious and moderate chronic liver disease patients, the virus is considered to be transmitted through blood transfusion or use of blood products. In Europe and the US, more than a half of blood products have been proved to contain genes of the said hepatitis viruses^(1,2,3,4). Chances of infection are higher for patients who have either direct or indirect contacts with blood. What deserves to be mentioned is that the virus has also been detected in saliva and feces^(9,10). Chances of infection via mouth and feces should not be ruled out. Some indirect and yet supporting evidence is the fact that in some African countries, though the infection rates were as high as 80%, blood transfusion was generally uncommon.

Liver functions of cases with TTV-DNA in their blood sera were not significantly abnormal⁽¹⁾. Though some researchers have reported that the existence of the virus could have some association with the time of onset of clinical symptoms; this finding, however, needs further study. There has never yet any significant direct evidence to date to indicate that the virus plays any role pathologically^(7,8,9). More studies are needed. However, by tracing the pathways of the virus molecules, the mechanism of infection of the virus can be understood. This understanding should help the promotion of public health programs.

The main purpose of the present study was to understand the infection of the virus among the general population of Taiwan and its likely routes of transmission. In present study, polymerase chain reaction was used to analyze the infection of TTV-DNA in healthy individuals and in hepatitis C patients, and its possible impact on the laboratory testing for liver functions. Lastly, gene sequencing method was used to establish the indigenous genotypes of the virus.

Materials and Method

Subjects for Study

Three groups were used for study. They were; 1) as the control group, 300 healthy individuals who visited the hospitals for routine health examinations; liver functions normal (average age 25.8 years), HBsAg negative, and anti-HCV negative; 2) 53 anti-HCV positive, ALT normal cases (36 males and 17 females, average age 34.5 years); and 3) 300 anti-HCV positive and ALT abnormal cases (148 males and 152 females, average age 36.4 years) (see Table 1). Specimens were collected by the Taipei Blood Center, Tainan Blood Center, Taipei Municipal Chung-hsiao Hospital, Ilan Lotung Po-ai Hospital, Changhua Christian Hospital, Pingtung Christian Hospital, and DOH Penghu Hospital.

In the study, ALT (alanine aminotransferase) was used to indicate liver functions. Its normal value was defined as a value lower than 45 U/L. Anti-HCV was first tested with the second generation Murex test reagent, and later with reagent of the Abbot Assay (second generation).

Detection of TTV-DNA

Materials used in the present study were serum specimens collected from blood centers and hospitals. Blood bags or serum specimens were sent to the Laboratory in frozen conditions, placed in bags and kept under -20°C . DNA was isolated directly from 100 μl plasma or serum with QIAamp Blood Kit (QIAGEN Ltd., Crawley, UK). The isolated DNA was placed in the 50 μl buffer solution. TTV-DNA was the only starting primer for the use of TTV cloned from the 50 μl PCR mixture (containing 5 μl of isolated DNA, 1xPCR buffer, 0.2 mM deoxynucleoside triphosphates, 2 mM magnesium chloride, 0.5 U Tag polymerase) with Perkin Elmer temperature circulator (PE 4800). The polymerase reaction was semi-nested. The primers used for the first cycle of cloning were: 5'-ACA GAC AGA GGA GAA GGC AAC ATG-3' (primer A; 10 pmol) 5'-CTG GCA TTT TAC CAT TTC CAA AGT T-3' (Primer B; 10 pmol). The primers used for the second cycle of cloning were: 5'-GGC AAC ATG TTA TGG ATA GAC TGG-3'(Primer C; 10 pmol) 5'-CTG GCA TTT TAC CAT TTC CAA AGT T-3' (Primer B; 10 pmol). Temperature changed in each cycle of cloning: 94°C , 30 seconds; 58°C , 30 seconds; and 72°C , 30 seconds, totaling 25 cycles. Positive specimens would have a synthetic product of 272 bp long. The product, after dying with ethidium bromide, could be observed under 2% agarose ultraviolet light. For the consistency of the PCR findings, primers were used repeatedly to test the negative cases in the control group.

In each testing, a serum specimen containing TTV-DNA (specimens verified by gene sequencing method, numbered W101) and another serum specimen not containing TTV-DNA (specimens verified repeatedly by PCR as negative, numbered W102) were used respectively as positive and negative controls (see Figure 1). A method developed by the authors was used to assess the amount of TTV-DNA in the specimen being about 10^7 - 10^8 molecules/ml⁽¹⁵⁾. The

sensitivity of the methods used for the detection of TTV-DNA was about 10-100 TTV-DNA molecules/ml.

Gene Sequencing Analysis

The cloned TTV-DNA's were processed for gene sequencing analysis after purification (QIA quick PCR purification kit; Qiagen Ltd., Germany). The sequencer used was the ABI 310 automated DNA sequencer (Applied Biosystems, Forster City, CA) with the fluorescent dye terminator cycle method.

Evolutionary Tree Analysis

The gene sequences of the indigenous TTV-DNA were compared with the 10 groups of gene sequences of the GenBank database (release 110); TTV-DNA sequences isolated in Japan and Europe also included. DNA sequences were compared with the Clustal W. method⁽¹⁶⁾. The neighbor-joining method was used to reconstruct the evolutionary trees⁽¹⁷⁾.

Statistical Method

The t-test of Sigma Plot was used. A *p*-value lower than 0.05 was considered statistically significant.

Results

Epidemiology

The length of the section obtained from the TTV-DNA by PCR method was about 272 bp (see Figure 1). By the finding, the TTV-DNA positive rate in sera of the Chinese population undergoing general health examinations was estimated to be 9% (27/300) (see Figure 1).

Association with Hepatitis C Patients

Both the anti-HCV and TTV-DNA positive rates can be seen in Table 1.

The positive rates of TTV-DNA in anti-HCV negative patients and anti-HCV positive patients were 9% and 31% respectively ($p < 0.05$) (see Table 1).

This finding suggested that TTV-DNA infection was, statistically, highly associated with hepatitis C virus infections.

Indigenous TTV Genotypes and their Distribution

To understand the sources and routes of transmission of the virus, DNA sequences of the virus was made by using tools of molecular biology to compare with DNA sequences of foreign virus strains, and thus to understand the mutation processes of the indigenous TTV. Cases of Taiwan TTV infection and the distribution of genotypes are shown in Table 2. Study findings showed that the major TTV strain of Taiwan was genotype I (about 73%). The subtypes were genotype IA (about 53%) and genotype IB (about 47%). Four new subtypes different from other already isolated TTV strains had been detected in the present study.

Discussion

TTV is a virus of high mutability. Since its identification in 1997, a large number of study reports on the mutated types of TTV and their geographic distribution have appeared. By the DNA sequencing analysis, the virus is found to be highly mutable in the ORF1 gene sequence ranges. Okamoto *et al.* reported that in this region of 200 bases, the mutability could be higher than 30%⁽¹⁰⁾.

In the process of virus detection, different primers produce different consistency and sensitivity. The primer used in the present study, ORF1, found a TTV-DNA positive rate of 9% (27/300) in the sera of the healthy control group. The TTV-DNA positive rates were 9% in the anti-HCV

negative and 31% in the anti-HCV positive cases ($p < 0.05$). The finding suggested that TTV-DNA infection could be associated with hepatitis C virus infection. The TTV-DNA positive rates in anti-HCV positive patients in Taiwan were found to be 37% by Kao *et al.*, and 15% by Ho *et al.* They were different from the 31% of the present study, possibly because different samples and geographic regions were studied. The conditions of liver functions were not associated with TTV infection (31% vs. 37%; $p > 0.05$). The worldwide major genotype of TTV is Genotype I. In the present study, the major genotype of TTV strains in Taiwan was also Genotype I (about 73%). The subtypes were Genotype IA (53%), and Genotype IB (47%). Some new subtypes had also been detected in the present study. They were significantly different from other already isolated TTV strains. As can be noted in the present study of certain groups, TTV infection seemed to be somewhat associated with hepatitis C virus infection. The detection of some new genotypes further confirmed the diversity of the virus reported from different countries. Further studies on these new genotypes are suggested.

Prepared by: Wang CH^{1,2}, Lin MH³, Yang TH⁴, Lo SH⁴, Chiu ZH³, Su CC³, Lu HC³, Su WW⁵, Shih MC⁵, Lee CC⁶, Chen CA⁷, Li L⁸, Lu SC⁹, Chen KL^{1,2}, Sabine Schleicher¹⁰, Bertram Flehmig¹⁰, Lu CF^{1,2}, Hsu HM²

1. Institute of Preventive Medicine, DOH
2. Center for Disease Control, DOH
3. Taipei Municipal Chung-hsiao Hospital
4. Lotung Po-ai Hospital
5. Changhua Christian Hospital
6. Pingtung Christian Hospital

7. DOH Penghu Hospital

8. Taipei Blood Center

9. Tainan Blood Center

Address: 115 No. 161, Kung-yang Street, Nankang District, Taipei

Tel: 02-2785-0513 ext 437

E-mail: chwang01@cdc.gov.tw

References

1. Nishizawa T, Okamoto H, Konishi K, et al. A novel DNA virus (TTV) associated with elevated transaminase levels in post-transfusion hepatitis of unknown etiology. *Biochem Biophys Res Com* 1997; 241: 92-97.
2. Simmonds P, Davidson F, Lycett C, et al. Detection of a novel DNA virus (TTV) in blood donors and blood products. *Lancet* 1998; 352: 191-195.
3. Okamoto H, Nishizawa T, Kato N, et al. Molecular cloning and characterisation of a novel DNA virus (TTV) associated with post-transfusion hepatitis of unknown etiology. *Hepatol Res* 1998; 10: 1-16.
4. Naoumov N, Petrova EP, Thomas MG, et al. Presence of a newly described human DNA virus (TTV) in patients with liver disease. *Lancet* 1998; 352: 195-197.
5. Hijikata M, Takahashi K, Mishiro S. Complete circular DNA genome of a TTV virus variant (isolate name SANBAN) and 44 partial ORF2 sequences implicating a great degree of diversity beyond genotypes. *Virology* 1999; 260: 17-22.
6. Abe K, Inami T, Asano K, et al. TTV virus infection is widespread in the general population from different geographic regions. *J Clin Microbiol* 1999; 37: 2703-5.

7. Charlton M, Adjei P, Poterucha J, et al. YY-virus infection in North American blood donors, patients with fulminant hepatic failure, and cryptogenic cirrhosis. *Hepatology* 1998; 28: 839-842.
8. Ikeda J, Takasu M, Inoue K, et al. Infection with an unenveloped DNA virus (TTV) in patients with acute or chronic liver disease of unknown etiology and in those positive for hepatitis C virus RNA. *J Hepatol* 1999; 30: 202-211.
9. Naoumov NV. TT-virus—highly prevalent but still in search of a disease. *J Hepatol* 2000; 33: 157-159.
10. Okamoto H, Nishizawa T, Ukita M. A novel unenveloped DNA virus TTV virus associate with acute and chronic non-A to G hepatitis. *Intervirology* 1999; 42: 196-204.
11. Okamoto H, Akahane Y, Ukida M, et al. Fecal excretion of a non-enveloped DNA virus (TTV) associated with post-transfusion non A-G hepatitis. *J Med Virol* 1998; 56: 128-132.
12. Ross RS, Viazov S, Runde V, et al. Detection of TTV virus DNA in specimens other than blood. *J Clin Virol* 1999; 13: 181-184.
13. Wang CH, Tschen SY. Affinity capture-polymerase chain reaction for quantitation of hepatitis B virus DNA. *Nucl Acids Res* 1994; 22: 4837-4839.
14. Thompson JD, Higgins DG, Gibson TJ, et al. Improving the sensitivity of progressive multiple sequence alignment through sequence weighting position-specific gap penalties and weight matrix choice. *Nucl Acids Res* 1994; 22: 4673-4680.
15. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing evolutionary trees. *Molecular Biology and Evolution*, 1987; 4: 406-425.
16. Kao JH, Chen W, Chen PJ, et al. TT virus infection in patients with

- chronic hepatitis B or C: influence on clinical, histological and virological features. *J Med Virol* 2000; 60: 387-92.
17. Dai CY, Yu ML, Chuang WL, et al. The epidemiology of TT virus (TTV) infection in a hepatitis C and B virus hyper-endemic area of southern Taiwan. *Kaohsiung J Med Sci* 2000; 16: 500-9.
18. Ho TF, Yang SC, Huang YT et al. TT virus infection in screened Taiwanese blood donors. *Vox Sang* 2000; 79: 198-200.
19. Simons JN, Leary TP, Dawson GJ, et al. Isolation of novel virus-like sequences associated with human hepatitis. *Nat Med* 1995; 1: 564-9.
20. Linnen J, Wages JJ, Zhang-Keck ZY, et al. Molecular cloning and disease association of hepatitis G virus; a transfusion transmissible agent. *Science* 1996; 271: 505-8.

Table 1 Positive Rates of TTV DNA in Hepatitis C Positive and Negative Patients

Groups	<i>TTV DNA</i> positive(%)	<i>TTV DNA</i> Negative(%)	No. of Patients	Average Age
Group 1: anti-HCV antibody negative; HbsAg Negative/ALT<45 U/L (the general healthy population)	27(9)	273(91)	300	25.8
Group 2: anti-HCV antibody positive; ALT<45 U/L (healthy population, once infected by hepatitis C virus, liver functions normal, no significant symptoms)	15(31)	38(69)	53	34.5
Group 3: anti-HCV antibody positive; ALT>45 U/L (hepatitis C patients, liver functions abnormal)	111(37)	189(63)	300	36.4

Figure 1 Electrophotograph of PCR Showing Consistency of TTVDNA

Specimens tested are in rows 1 through 5 (rows 1, 2, 4 are negative; rows 3 and 5 are positive); row 6 is the positive controls (No. W101); row 7 is the negative controls (No. W102); row M is the DNA standard marker (DNA Standard VI Marker, Boehringer Mannheim, Germany).

M 1 2 3 4 5 6 7

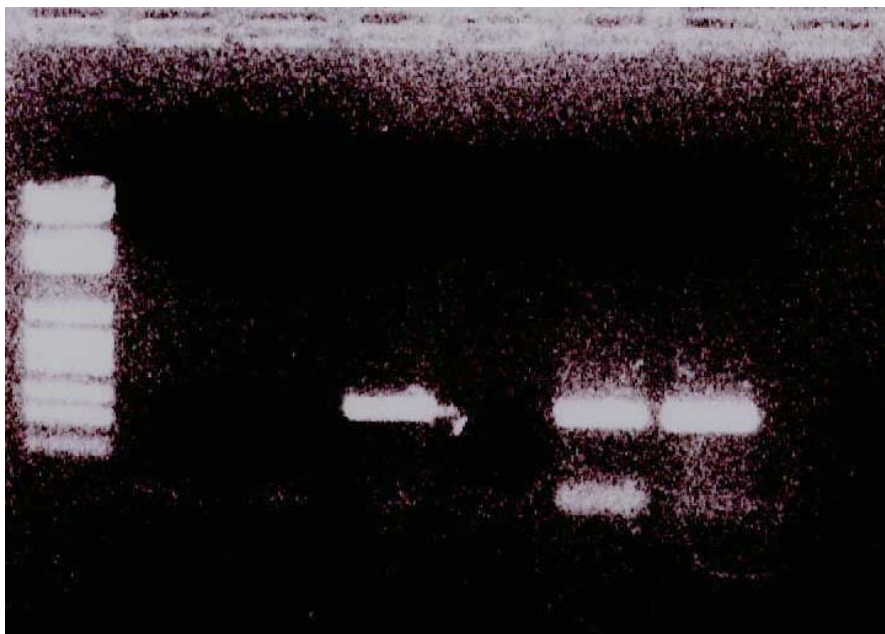


Figure 2 Distribution of TTV in Hepatitis C Positive and Negative Groups

Group 1 : anti-HCV antibody negative; HbsAg negative/ALT<45 U/L (the healthy general population)

Group 2 : anti-HCV antibody positive; ALT<45 U/L (healthy general population once infected with hepatitis C, liver functions normal, and no symptoms)

Group 3 : anti-HCV antibody positive; ALT>45 U/L (hepatitis C patients, liver functions abnormal)

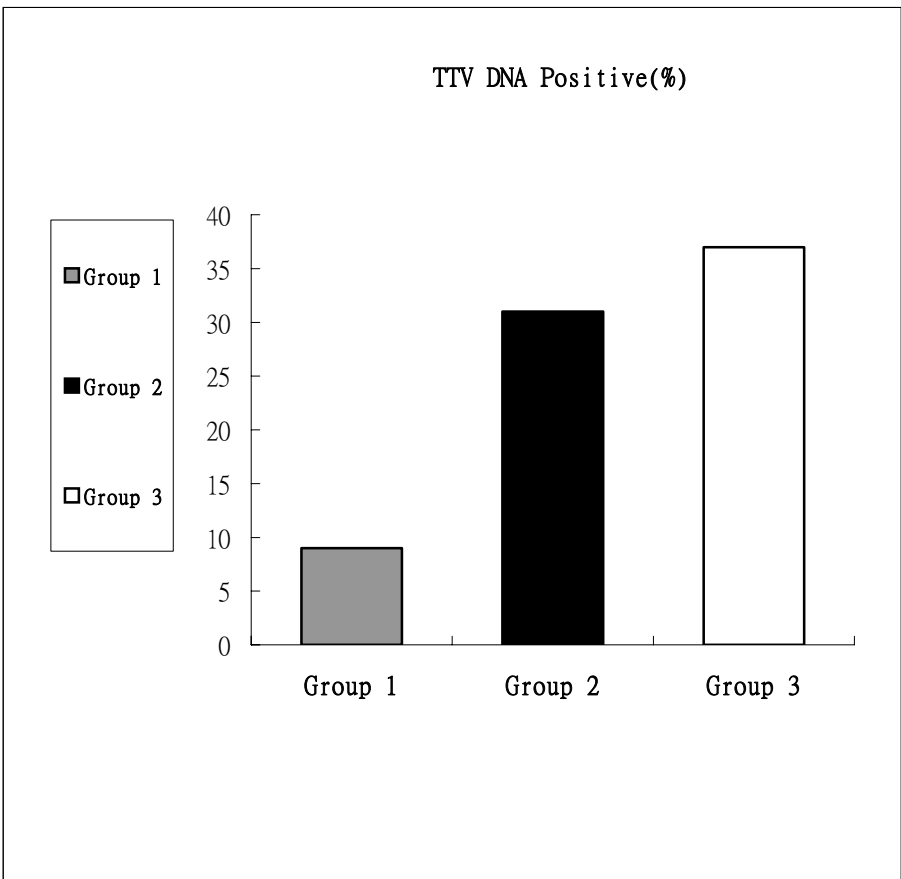


Table 2 Cases of TTV Infection and Distribution of Genotypes

Notes: F: Female; M: Male; Ia/Ib: between genotypes Ia and Ib; none: no data of this genotype available in the GenBank databases.

No. of Patients	Age	Sex	ALT	No. of Virus	TTV type	Similar with the Genebank
TTV89-001	22	M	35	TW16	Ib	AF07273820
TTV89-002	21	M	27	TW17	Ib	AB011494
TTV89-003	21	F	20	TW27	IId	None
TTV89-004	23	M	10	TW29	IId	None
TTV89-005	37	M	11	TW28	IId	None
TTV89-006	22	M	39	TW30	IId	None
TTV89-007	29	F	26	TW21	Ia	AF055897
TTV89-008	21	F	22	TW22	Ia	AB011487
TTV89-009	18	M	26	TW19	Ia/Ib	AB008394
TTV89-010	39	F	11	TW20	Ia/Ib	AB008394
TTV89-011	18	M	15	TW25	Ia/Ib	AB008394
TTV89-012	23	F	13	TW26	Ia/Ib	AB008394
TTV89-013	28	F	10	TW24	Ia/Ib	AB008394
TTV89-014	22	M	17	TW23	Ia/Ib	AB008394
TTV89-015	26	F	7	TW18	Ib	AB011491