Transfusion-transmitted virus prevalence in subjects at high risk of sexually transmitted infection in Turkey

M. Yazici[1], M. R. Cömert[1], R. Mas[1], C. Guney[2], E. Cinar[3] and I. H. Kocar[1]

Departments of [1]Internal Medicine, [2]Virology and [3]Blood Bank, Gülhane School of Medicine, Ankara, Turkey

Objective To assess the possible sexual transmission of virus and to identify the prevalence of TTV viremia in Turkey and its association with other hepatotropic viruses.

Methods Serum samples were collected from 81 subjects (74 prostitutes and seven homosexual men) at high risk of sexually transmitted infection and from 81 healthy controls (74 females and seven males). Sera of patients and controls were tested for TTV, hepatitis A virus, hepatitis B virus, hepatitis C virus, and human immunodeficiency virus. Also, serum alanine and aspartate aminotransferases were measured.

Results The prevalence rates of TTV viremia in the risk group and control group were 86.4% and 82.7%, respectively. There was a statistical difference in mean age between TTV-infected and uninfected subjects (38.6 ± 9.9 versus 32.2 ± 6.1 years, respectively, $P < 0.001$). Prevalence rates of TTV infection in subjects with positive anti-HAV and positive anti-HBc were high when compared with subjects who were negative for these.

Conclusion We suggest that TTV infection has a diverse route of transmission, and its prevalence increases with age; also, the prevalence rate of TTV is high in certain risk groups. The prevalence rates of TTV in the group at risk for sexual transmission (86.4%) and in the control group (82.7%) were among the highest ever reported in the world. Also, we suggest that TTV generally does not cause clinical disease, in spite of this high prevalence.

Keywords TTV, sexual transmission, prostitutes, homosexual men

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INTRODUCTION

Transfusion-transmitted virus (TTV) is a DNA virus that was discovered in three of five patients with cryptogenic post-transfusion hepatitis by Nishizawa et al. [1]. TTV has a high buoyant density ($1.26\,g/cm^3$), single-stranded DNA that consists of at least 3700 bases, and no outer envelope [2]. These features of TTV resemble those of the Parvoviridae, but TTV shows no sequence homology with the Parvoviridae [3]. More recent studies have shown that TTV has a diameter of 30–50 nm, a circular, single-stranded DNA genome, and a density in caesium chloride of 1.31–1.34 g/cm^3 [3]. These findings suggest that TTV resembles the Circoviridae, which infect plants and vertebrates [3].

In the past, transmission of virus was believed to occur primarily by transfusion as well as other parenteral routes. In the course of time, other transmission routes, such as sexual, maternal, and fecal–oral, and other contact, have been demonstrated [4–9].

The reported seroprevalence of TTV in different countries and in different risk groups varies greatly. Reports from developed countries, such as North America, Scotland and Japan, have shown that the prevalence in healthy populations is 1–2%,

Corresponding author and reprint requests: B. Cömert, Gülhane School of Medicine, Department of Internal Medicine, Etlik, 06018, Ankara, Turkey
Tel: +90 312 3043060
Fax: +90 312 3042150
E-mail: bcomart@ttnet.net.tr

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whereas the prevalence is about 83% in rural populations of Asian, African and South American developing countries [4,5,10,11]. Another reported feature of TTV prevalence is its correlation with increasing age [5,6,12]. In contrast, in some other studies, no relationship was found between age and TTV positivity in different study populations [13–15].

The first aim of this study was to assess the effect of possible sexual transmission of virus on the prevalence of TTV among prostitutes and homosexual men. Second, we aimed to identify the prevalence of TTV viremia and its association with other hepatotropic viruses in the central Anatolia region of Turkey.

MATERIALS AND METHODS

Serum samples were collected from 81 subjects (74 prostitutes and seven homosexual men; mean age 38, range 24–65) at high risk of sexually transmitted infection. Seventy-four females and seven males (mean age 37, range 21–62) who requested blood grouping at our hospital blood bank were included as a control group. Serum samples were stored at −70 °C until they were used. Homosexual men of our study group consisted of male prostitutes. During the enrollment of the control group to the study, the subjects were asked how many sexual partners they had had until that time. The candidates for the control group were informed about the study and asked not be truthful about the number of their sexual partners. Those who had had only one sexual partner were included in the study. Also, a Veneral Disease Research Laboratory (VDRL) test was performed and questions about genital symptoms were asked, to exclude possibility of sexually transmitted disease (STD). Neither the sexual transmission risk group (STRG) nor the control group had a history of intravenous drug use.

Sample preparation and detection of TTV DNA

Sera of patients and controls (60 μL) were pretreated with pronase E and its buffer at 40 °C for 60 min, and then nucleic acids were extracted by the alkali–phenol and chloroform method. Extracted nucleic acids were dissolved in 20 μL of DNase- and RNase-free deionized distilled water and then subjected to PCR as described previously [16]. In brief, the thermal cycler was programmed to preheat at 95 °C for 10 min to denature sample DNA, and then samples (10 μL of extracted DNA mixed with 2.0 mM $MgCl_2$, 30 pmol of each primer, sense and antisense, 2 U of *Taq* polymerase, 10 mM dNTP) were subjected to 45 cycles consisting of 94 °C for 20 s, 60 °C for 20 s, and 72 °C for 30 s in an MJ Research thermal cycler (MJ Research Inc., Waltham, MA, USA). The sequences of the TTV-specific primers from 5′-UTR were T801 (5′-GCb ACG TCA CTA ACC ACG TG-3′, sense primer, nucleotides 6–25) and T935 (5′-CTb CGG TGT GTA AAC TCA CC-3′, antisense primer, nucleotides 204–85; b = G, C or T), as designed by Takahashi et al. [16]. Multiple positive and negative controls were run in each PCR assay. Positive controls, which had been obtained and stored at −80 °C in the previous studies, were used. Also, the gene region used for PCR cloned to PCR 2.1 plasmid for determination of sensitivity and it was used as a positive control. The sensitivity of PCR was determined as 10–100 copy/mL in a PCR–TTV sensitivity test performed with cloned TTV plasmid (PCR 2.1 + TTV Amplicon, Topo TA cloning kit, Invitrogen, Carlsbad, CA, USA). PCR products (199 bp in length) were analyzed by 2% agarose gel electrophoresis after ethidium bromide staining, and photographed under UV light.

Detection of other virologic markers

Each sample was tested for anti-HAV IgG by ELISA (Bioelisa HAV, Biokit, Barcelona, Spain). In addition, each sample was tested for HBsAg (Murex HBsAg Version 3, Abbott/Murex, Dartford, UK), anti-HBc IgG (Wellcozyme anti-HBc, Abbott/Murex), anti-HCV (Innotest HCV Ab IV, Innogenetics, Gent, Belgium), and anti-HIV 1/2 (Innotest HIV-1/HIV-2 Ab sp., Innogenetics, Belgium), by enzyme immunoassay.

Assays of aminotransferases

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured by autoanalyzer (Hitachi 917, Roche Diagnostics, Mannheim, Germany).

Statistical analysis

The Mann–Whitney *U*-test, Student's *t*-test, the chi-square test, Fisher's Exact Test or the logistic

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Table 1 General features and viral serologic results of groups

	STRG $n=81$	Control group $n=81$	*P*
Male/Female	7/74	7/74	
Age (mean ± SD years)	38.2 ± 9.8	37.0 ± 9.7	>0.05[a]
Positive TTV DNA	70 (86.4%)	67 (82.7%)	>0.05[b]
Positive anti-HAV	48 (59.3%)	45 (55.6%)	>0.05[b]
Positive HbsAg	5 (6.2%)	4 (4.9%)	>0.05[c]
Positive anti-HBc	53 (65.4%)	29 (35.8%)	<0.001[b]
Positive anti-HCV	1 (1.2%)	1 (1.2%)	
Positive anti-HIV	0	0	

[a]*t*-test; [b]chi-square test; [c]Fisher's Exact Test. STRG, sexual transmission risk group.

Table 2 Comparison of TTV DNA-positive and -negative study subjects

Risk factors	*n*	TTV DNA Positive	TTV DNA Negative	*P*
Age (mean ± SD, years)	137/25	38.6 ± 9.9	32.2 ± 6.1	=0.002[a]
STRG	70/11	39.1 ± 9.9	32.3 ± 6.4	=0.031[b]
Control group	67/14	38.0 ± 10.1	32.1 ± 6.1	=0.038[a]
Positive/negative anti-HAV	93/69	85/52	8/17	=0.005[c]
STRG	48/33	45/25	3/8	=0.043[d]
Control group	45/36	40/27	5/9	>0.05[c]
Positive/negative anti-HBc	82/80	76/61	6/19	=0.004[c]
STRG	53/28	50/20	3/8	=0.007[d]
Control group	29/52	26/41	3/11	>0.05[d]

[a]Mann–Whitney *U*-test; [b]*t*-test, [c]chi-square test; [d]Fisher's Exact Test. STRG, sexual transmission risk group.

regression test were used to analyze the data where appropriate. A *P*-value of less than 0.05 was considered significant.

RESULTS

TTV DNA was detected in 70 (86.4%) of 81 subjects (prostitutes 64/74, homosexual men 6/7) (Table 1). Of the 81 control subjects, 67 (82.7%) had positive results for TTV DNA (females 62/74, males 5/7). The difference in the prevalence rates of TTV viremia between the STRG and control group was not statistically significant ($P > 0.05$). There was no statistical difference in age between these two groups (STRG 38.2 ± 9.8 years; control group 37.0 ± 9.7 years). However, there was a statistical difference in age between TTV-infected and uninfected subjects (38.6 ± 9.9 versus 32.2 ± 6.1 years, $P = 0.002$, Table 2). Logistic regression analysis showed that TTV prevalence increases with increasing age ($r = 0.1894$, $P < 0.05$).

The prevalence rates of positive anti-HAV results in both groups were similar (STRG, 48/81 (59.3%); controls, 45/81 (55.6%); $P > 0.05$; Table 1). The TTV prevalence rates in the STRG and in all study subjects (STRG plus control group) with positive anti-HAV were statistically higher than in those with negative anti-HAV ($P = 0.043$ and $P = 0.005$, respectively; Table 2). The prevalence rates of positive anti-HBc results in both groups were statistically different (65.4% versus 35.8%; $P < 0.001$; Table 1). The TTV prevalence rates in the STRG and in all study subjects with positive anti-HBc were statistically higher than in those with negative anti-HBc ($P = 0.007$ and $P = 0.004$, respectively; Table 2).

A positive anti-HCV result was obtained in one subject each from the STRG and control group. However, HCV RNA assays in both cases were negative with PCR. None of the subjects from the STRG or control group had anti-HIV. Serum ALT and AST levels were normal in all study subjects, but not in one subject (ALT 54 U/L; AST 82 U/L). This 31-year-old-prostitute had positive TTV only.

DISCUSSION

When Nishizawa et al. first described TTV in patients with cryptogenic post-transfusion hepatitis [1], it was believed that virus transmission was

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via transfusion. However, more recent studies have shown that there are diverse routes of transmission of TTV [4–9].

In our study, the prevalence rate of TTV in a sexual transmission risk group was slightly higher than in controls, but the difference was not statistically significant (86.4% versus 82.7%). Our finding was in agreement with those of MacDonald et al. [12], who also found no statistical difference between patients at risk for sexual transmission (52 prostitutes, 81 homosexual men) and normal controls, but their prevalence rates (13.4% and 9.8% versus 4.5%, $P > 0.05$) were very low when compared with ours. On the other hand, Huang et al. reported that the prevalence rate of TTV in prostitutes was significantly higher than in the control group (32.9% versus 21.3%) [13]. In our study, the prevalence rate of TTV in normal control subjects was one of the highest ever reported (82.7%). Previous reports on prevalence from different countries indicate that the prevalence is high in the developing countries [4,5,10,11]. In another study, TTV seroepidemiology was assessed in individuals, including healthy populations and different risk groups from ten different geographic regions [17]. The prevalence rates found in that study varied from 70% to 100%. The probable reason for the very different results obtained in prevalence studies performed in the same geographic area with similar groups is the type of primer used in the TTV PCR tests. Previous techniques had low sensitivity and unstable reactions. To overcome this problem, a more sensitive and stable new primer combination was developed by Takahashi et al. [16]. Using their new PCR system, they reported that the prevalence of TTV in healthy individuals in Japan was 92% [16].

The prevalence rates of other hepatotropic viruses, but not hepatitis B virus (HBV), were similar in both groups. The prevalence rate of HBV was significantly higher in the STRG. This high prevalence showed the efficacy of the sexual transmission route for HBV.

We found that the mean age of subjects who were TTV positive was significantly higher than that of subjects who were TTV negative, and that TTV prevalence increased with advanced age. This finding was in agreement with many reports showing that the TTV prevalence rate was high in older age groups [5,6,12]. In contrast, there are reports showing no association between age and TTV viremia [13–15]. The lack of relationship between age and TTV positivity in these studies may be explained by the low prevalence of TTV in the populations studied.

Our results showed significantly higher prevalence rates of TTV infection in subjects positive for anti-HAV and anti-HBc when compared to subjects with negative anti-HAV and negative anti-HBc (91.4% versus 75.4%, and 92.7% versus 76.3%, respectively). This high prevalence of virus in subjects with anti-HAV and anti-HBc suggests that TTV may share common modes of transmission with HAV and HBV. According to our results, transmission of TTV via sexual contact was not as efficient as transmission of HBV in our study population. However, these findings suggest that sexual transmission has at least a minor role in TTV transmission.

The prevalence rate of hepatitis C virus (HCV) in our study subjects was very low, and we were not able to establish any association between TTV and HCV infection. However, Kao et al. showed an increased prevalence of TTV infection in patients with chronic HCV infection [18]. Another report from Japan also showed an increased frequency of TTV viremia in patients with HCV infection [10].

All of the subjects, except for one prostitute, had no biochemical evidence of liver disease. This finding suggests that TTV, like the hepatitis G virus, has no clear disease association. Similarly, there are some reports showing TTV viremia to have no relationship to liver disease or hepatocellular carcinoma [4,10,19,20]. On the other hand, Charlton et al. found an increased prevalence of TTV in patients with liver disease, including cryptogenic cirrhosis and fulminant hepatic failure [4]. Christensen et al. investigated the pathogenic role of TTV in patients with human immunodeficiency virus. They found an association between high TTV viremia and decreased survival, and suggested that TTV may be an opportunistic pathogen [21].

In one study, vertical transmission of TTV was assessed and 19% of cord bloods were found to be TTV infected [6]. TTV viremia in 68 children was assessed in plasma samples collected in the 3rd and 12th months after birth in a rural area of the Democratic Republic of Congo [14]: 37 children became TTV infected after 3 months of age, while TTV viremia was detected in only two children in the 3-month samples. These data suggest that an environmental source of TTV infection, rather than vertical transmission seems to be more likely.

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Okamoto et al. showed that TTV transmission via the fecal–oral route is also possible [7]. They detected TTV DNA in feces from three of five patients with post-transfusion non-A–G hepatitis.

All of these data, as well as our own, indicate that TTV infection has diverse routes of transmission, its prevalence increases with age, and the prevalence rate of TTV is high in certain risk groups such as patients with hepatitis A–E, prostitutes and homosexual men. Although the prevalence rates of TTV in developing or underdeveloped countries are higher than in developed countries, TTV viremia is widespread in the world. The TTV prevalence rate in normal controls was found to be very high (82.7%) in the central Anatolia region of Turkey. There was no obvious evidence of clinical disease in spite of this high prevalence rate.

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