

# Presence and significance of transfusion-transmitted virus infection in Iranian patients on maintenance hemodialysis

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**Background and Purpose:** Transfusion-transmitted virus (TTV), a recently discovered DNA virus, was first identified in patients with non-A to -G hepatitis following blood transfusion. Transmission is generally via the parenteral route but recent data suggest that TTV can also be transmitted by the fecal-oral route.

**Methods:** This cross-sectional study was conducted in March 2005 and included 324 patients on maintenance hemodialysis (HD) at 3 different centers in Tabriz, Iran. Demographic and clinical data were recorded. Blood samples for virological and biochemical tests were drawn simultaneously. TTV DNA was detected using semi-nested polymerase chain reaction. Serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase were also measured.

**Results:** Overall seroprevalence of TTV was 9.3% (95% confidence interval, 6.1-12.5%). Prevalence rates of hepatitis B surface antigen, hepatitis C virus antibody, and hepatitis E virus antibody were 4.6% (15/324), 20.4% (66/324), and 7.4% (24/324), respectively. Patients were negative for human immunodeficiency virus antibody. There was no association between TTV infection and elevated ALT levels. TTV-positive patients were significantly younger than TTV-negative patients ( $p=0.018$ ). There was no significant association between TTV positivity and age, gender, duration of HD, positivity for hepatitis B, C, or E virus infection markers, and history of transfusion and transplantation.

**Conclusion:** We observed low TTV prevalence and no association between TTV and blood-borne infections in our HD patients. TTV infection was not related to elevated levels of liver enzymes; however, the clinical impact of this virus need further investigations.

**Key words:** Dialysis; Hepatitis; Iran; Polymerase chain reaction

## Introduction

A novel DNA virus, transfusion-transmitted virus (TTV), was identified in 3 Japanese patients with post-transfusion non-A to -G hepatitis in 1997 [1]. TTV is a non-enveloped single-stranded DNA virus (genome length of approximately 3.7 kb) that was initially thought to be distantly related to parvoviruses [2] or circoviruses [3]. However, recent molecular and biophysical characterization of TTV indicates that this virus has a

circular negative-strand DNA genome and could therefore constitute a new virus family [4].

TTV is found in many body fluids and secretions, including stools, saliva, semen, breast milk, and tears [5]. TTV is generally transmitted by the parenteral route [2,6]; recent data suggest that TTV can also be transmitted enterally [7]. Furthermore, vertical and sexual transmissions have been reported [8].

The frequency of TTV infection in patients on maintenance dialysis varies widely. In hemodialysis (HD) populations, TTV prevalence rates range from 2% to 53% [9-12]. The geographical distribution, TTV DNA detection methods, study group size, and the

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presence of various demographic or clinical features of the HD patients all account for the differences.

The pathology of TTV infection has not yet been elucidated. Association between TTV infection and acute or chronic hepatitis or other diseases has not been consistently observed [13]. Although it has been shown that TTV titers correlate with elevation in serum aminotransferase concentrations, the initial observation of hepatotropism has not been confirmed [14] since injection of the virus into chimpanzees, while capable of causing infection, did not result in clinical illness [4].

Patients on long-term HD are especially susceptible to parenterally transmitted agents and therefore represent an important population for analysis of the clinical and epidemiological implications of newly identified agents.

In the present study, we analyzed all patients on maintenance HD in dialysis units at 3 university hospitals in Tabriz for the presence of TTV infection. Potential risk factors associated with TTV transmission and possible relationships with medical, biological, and epidemiological markers were also investigated.

## Methods

### Patients

This study was carried out in March 2005 and included all 324 patients (190 males and 134 females) on maintenance HD at 3 different dialysis units in Tabriz, Iran. The following clinical variables were registered from the medical records of patients: date of birth, gender, history of intravenous drug abuse, etiology of renal disease, history of jaundice, duration of HD, history of renal transplantation, and the total number of blood transfusions. Routine dialysis techniques (3- or 4-h treatments) were performed 3 times a week.

### Laboratory assay

Blood samples for detection of TTV were drawn before planned HD. Immediately after the collection of blood samples, serum was separated and stored at  $-20^{\circ}\text{C}$ . Coded sera were transferred to Tehran and further tested at the Infectious Diseases and Tropical Medicine Research Center. Serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were also measured.

All patients were previously tested for hepatitis B surface antigen (HBsAg) using enzyme-linked immunosorbent assay (ELISA; DiaSorin, Stillwater, MN, USA), anti-hepatitis C virus (HCV; ELISA, DiaSorin), anti-

hepatitis E virus (HEV; Enzyme Immunoassay, Dia.Pro Diagnostic Bioprobes, Milano, Italy), and anti-human immunodeficiency virus (HIV; ELISA, Biotest, Dreieich, Germany).

### Detection of TTV by polymerase chain reaction

DNA was extracted from serum samples (100  $\mu\text{L}$ ) by using a DNA extraction kit (QIAGEN Ltd., Crawley, UK), in accordance with the manufacturer's protocol. To avoid contamination, DNA extraction was performed in a separate room. In addition, water was extracted and amplified in each assay as a negative control.

DNA was first pre-warmed at  $95^{\circ}\text{C}$  and then quickly chilled on ice. TTV DNA was amplified by semi-nested polymerase chain reaction (PCR) with TTV-specific primers derived from 2 conserved regions based on the published sequences NG059, NG061, and NG063 [2].

PCR was performed using 5  $\mu\text{L}$  template DNA in 25  $\mu\text{L}$  reaction mixtures containing PCR buffer, 2 mM magnesium chloride, 0.5 mM of each primer, 0.2 mM dNTP, and 0.3 U Taq polymerase. Reactions were performed in an Eppendorf, Mastercycler® gradient 5331 (Eppendorf AG, Hamburg, Germany) using the following program steps: 1 min denaturation at  $95^{\circ}\text{C}$ , then 35 cycles at  $94^{\circ}\text{C}$  (30 sec),  $60^{\circ}\text{C}$  (45 sec), and  $72^{\circ}\text{C}$  (45 sec), followed by a final extension for 5 min at  $72^{\circ}\text{C}$  with NG059 as the sense primer and NG063 as the antisense primer. Negative and positive controls were included for every 5 and 10 reactions, respectively.

The second set of reactions was the same as the first set except that 5  $\mu\text{L}$  of the product from the first set of reactions was used as the template with NG061 and NG063 primers. The amplification was carried out as follows: denaturation for 1 min at  $95^{\circ}\text{C}$ , then 25 cycles at  $94^{\circ}\text{C}$  (30 sec),  $60^{\circ}\text{C}$  (45 sec), and  $72^{\circ}\text{C}$  (45 sec), followed by a final extension for 5 min at  $72^{\circ}\text{C}$ .

PCR products were separated by 2% agarose gel electrophoresis, stained with ethidium bromide, and photographed under ultraviolet light. All positive samples were confirmed by retesting.

### Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) for Windows (Version 11.5; SPSS, Chicago, IL, USA). Descriptive statistics were reported. Quantitative variables were expressed as mean  $\pm$  standard deviation (SD), and comparisons were performed using the two-sample *t* test.

Chi-squared test and Fisher's exact test were used to compare the proportions between groups. Statistical significance was set when  $p < 0.05$ .

### Ethics

This study was formally approved by the ethics committee and all participating patients gave their written informed consent.

### Results

Serum TTV DNA was detected by semi-nested PCR in 30 (9.3%) of 324 patients on maintenance HD. This prevalence is significantly higher than that in blood donors from the same geographical area where TTV was detected in 11 (2.7%) of 407 individuals ( $p < 0.001$ ) [unpublished data].

The clinical and demographic characteristics of the 30 uremic patients with TTV infection and the 294 uninfected patients are shown in Table 1. The prevalence rate for anti-HCV, HBsAg, and anti-HEV were 20.4% (95% confidence interval [CI], 16-24.8%), 4.6% (95% CI, 2.3-6.9%), and 7.4% (95% CI, 4.6-10.6%), respectively.

The median time on HD prior to entry in the study was 27 months (range, 1-261 months). The duration of HD was  $53.45 \pm 15.11$  (mean  $\pm$  SD) months.

The presence of TTV DNA was found to be related to the age of the patients. TTV-positive patients were significantly younger than TTV-negative patients ( $p < 0.018$ ). The mean age of TTV-positive patients was  $47.27 \pm 13.66$  years and that of the TTV-negative patients was  $54.08 \pm 15.13$  years. There was no significant difference in gender, duration of HD, anti-HCV-positive rate, HBsAg, anti-HEV rate, and AST/ALT levels between patients with and without TTV infection.

The etiology of end-stage renal disease necessitating maintenance HD was as follows: glomerulonephritis (34.9%), diabetic nephropathy (22.5%), chronic interstitial nephritis (1.5%), angiosclerosis (8.6%), hypertensive nephropathy (2.5%), polycystic kidney disease (6.8%), unknown etiology (21.6%), and miscellaneous (1.5%).

### TTV, blood transfusions, and renal transplantation

111 of 324 patients (34.3%) had a history of blood transfusion during the previous 6 months (range, 1-80 units). To further evaluate the possible risk factors for TTV infection, we performed chi-squared analysis of the clinical and demographic characteristics of the 111 patients with definite transfusion and 34 patients with transplantation history. Of the 111 patients who had received transfusion, 13 patients were found to be TTV positive, and the TTV-positive rate in patients with a previous transfusion was 11.7%. Among patients without any history of transfusion, 8% were TTV positive; however, this was not found to be significant.

Renal transplantation was carried out in 34 patients (10.5%). Of the 34 transplantation patients, 6 were infected with TTV (17.6%). There was no significant difference in TTV viremia between transplanted and non-transplanted patients ( $p = 0.108$ ).

### Discussion

A new DNA virus named TTV was identified in 1997 in Japan from the serum of patients with post-transfusion non-A to -G hepatitis. The discovery of another virus potentially causing liver disease induced us to study the TTV seroprevalence in patients at high risk for parenterally transmitted agents. Currently, no data is

**Table 1.** Demographic and clinical characteristics of patients with and without transfusion-transmitted virus (TTV) DNA

Characteristic	TTV DNA		<i>p</i>
	Positive (n = 30)	Negative (n = 294)	
Age (years; mean $\pm$ SD)	47.27 $\pm$ 13.66	54.08 $\pm$ 15.13	0.018
Male	22	168	NS
Female	8	126	NS
Duration of HD (months; mean $\pm$ SD)	39.63 $\pm$ 39.59	37.51 $\pm$ 37.39	NS
HCV Ab-positive rate (no. [%])	4 (13.33)	62 (21.08)	NS
HBsAg-positive rate (no. [%])	2 (6.66)	13 (43.33)	NS
AST (IU/L; mean $\pm$ SD)	9.13 $\pm$ 10.93	9.78 $\pm$ 15.42	NS
ALT (IU/L; mean $\pm$ SD)	9.50 $\pm$ 11.29	10.26 $\pm$ 16.30	NS

Abbreviations: SD = standard deviation; HD = hemodialysis; HCV Ab = hepatitis C virus antibody; HBsAg = hepatitis B surface antigen; AST = aspartate aminotransferase; ALT = alanine aminotransferase; NS = not significant ( $p > 0.05$ )

available on TTV infection among hemodialyzed patients in Iran.

In HD populations, the TTV prevalence rates range between 2% and 53% [9-12]. Our data show that TTV is not common among our HD patients with a prevalence of 9.3%; this percentage is lower than what was reported in a previous Japanese study [11]. The high prevalence of TTV infection among Japanese patients on HD may reflect the relatively high prevalence in the general population. A much lower prevalence of infection was found in a series from France in which TTV DNA was detected in only 2% of patients on HD [9]. A possible explanation may be the difference in the pattern of spread of infection between geographic areas. Moreover, taking into account the existence of previously described TTV genotypes, the geographic and genotypic prevalence may underestimate the real diffusion of infection in our area [15-17].

Evidence also suggests that the PCR method used may greatly affect the results of prevalence studies. The fact that our primers were those used by Okamoto et al [2] might, however, have contributed to the low prevalence rate detected. A recent study by Takahashi et al [18] showed that the proportion of TTV carriers among healthy Japanese could be as high as 92%. Thus, more standardized detection methods are needed to establish the true prevalence worldwide.

The association between TTV infection and hepatitis is controversial [19]. The virus was first suspected as being a pathogen when it was detected in patients with fulminant hepatitis of unknown etiology. In subsequent studies, it was shown that the virus did not have an important pathogenicity in TTV-positive healthy cases and in different subgroups. Concomitant infection with TTV and either HBV or HCV is common [20]. However, the effect of TTV infection in cases of chronic HBV or HCV infection is unknown. Kao et al [20] reported that coinfection with TTV did not affect the clinicopathological course of chronic hepatitis B or C or the response to interferon-alpha therapy. No significant relationship was found between TTV viremia and HBV, HCV, or HEV in our patients.

As far as the clinical impact of TTV infection is concerned, our data show that abnormal liver tests were uncommon in HD patients infected with TTV alone, in contrast to patients infected with known hepatotropic viruses such as HBV and HCV. Thus, our results do not support a role for TTV as a causative agent of liver disease in HD patients. However, our data do not exclude the possibility that mild and transient abnormalities in

liver enzymes detected in a proportion of HD patients with elevated aminotransferase and positive TTV DNA but negative for HBsAg and anti-HCV were caused by TTV infection. It is noteworthy that PCR methodology permits the detection of only the active forms of infection and that some patients may have been infected by TTV and may have recovered. Infection in such patients could only be confirmed by a serological method, but these have not yet become available.

In accordance with previous studies, no relation between TTV infection and the duration of HD was found [12,21]. Two studies [10,11] found that patients on maintenance HD became infected soon after the initiation of dialysis, suggesting that whether or not a patient is receiving HD is a more important factor than the duration of dialysis.

We could not demonstrate any relationship between TTV viremia and either the total number of blood transfusions or history of transplantation. TTV infection in the non-transfused patients might be related to dialysis or to a non-parenteral route, as has been previously described. In fact, the discovery of TTV excretion in the feces suggests that the infection may be transmitted by the fecal-oral route [7]. Moreover, the importance of non-parenteral transmission of TTV is supported by the higher percentage of infection than other hepatitis viruses among blood donors [22,23]. The fact that HD patients are polytransfused makes it likely that they are at risk of multiple exposures.

We also found that TTV-positive viremia was more frequent in younger patients. It is suggested that TTV may be acquired early in life, particularly in countries with a high prevalence of infection in the general population. Moreover, the observation that 63% of children aged 4 years were viremic suggests that acquisition of infection early in life is frequent [24]. In contrast, some studies have shown that increasing age is the strongest predictor of TTV positivity.

In conclusion, TTV was not prevalent in our patients on maintenance HD. However, it must be taken into account that the epidemiological relevance of TTV infection is probably underestimated due to the difficulty in detecting the corresponding antibody. Whether blood transfusions increase the risk of TTV infection was not revealed by this study. Our data did not show that the presence of TTV alone induces alterations in liver function tests. However, in TTV-positive patients, long-term follow-up is necessary in order to clarify the effects of TTV on liver function. Also, the involvement of TTV in human pathology and its relationship with the host

immune system remains unclear and warrants further investigations.

Since we have shown that TTV infection is acquired early in life and that recognizing pathologies produced in a fraction of infected individuals can be extremely difficult, further investigations should focus on infants to identify primary infections and possible associated diseases.

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