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Renal Data from the Arab World

Transfusion-transmitted Virus Infection in Hemodialysis Patients in Arar, Saudi Arabia: Prevalence, Predictors and Genotyping

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ABSTRACT. Transfusion-transmitted virus (TTV) is a single-stranded DNA virus that was identified in patients with post-transfusion hepatitis of non-A-to-G type. Patients with chronic renal failure on maintenance hemodialysis (HD) have a higher risk of viral infections, and the prevalence of TTV infection is common. The aim of our study was to detect TTV-DNA and its genotype in HD patients. A case–control study compromising of 63 patients on maintenance HD therapy at the Nephrology Center of Central Arar Hospital and 100 healthy individuals who were tested for TTV-DNA and its genotype by semi nested-polymerase chain reaction with primers derived from the conserved open reading frame 1 (ORF1) region followed by digestion with NdeI and PstI restriction enzyme. The results show that the prevalence of TTV in HD patients was high and statistically significant; 42.9% compared with 19% in the control group. History of blood transfusion was the only significant predictor, and we found that age of patients, duration of HD, hepatitis B and C infection, aspartate aminotransferase and alanine aminotransferase levels were not significant predictors of TT virus positivity in HD patients. TTV genotype 1 (G1) was found to be the most common genotype among both HD and healthy controls. The prevalence of TTV among HD patients was significantly higher than that in healthy individuals. History of blood transfusion was the only significant predictor of TTV positivity among them. Genotype 1 was the most predominant type among HD and healthy individuals. Further studies on TTV in peritoneal dialysis patients and transplant patients are needed.

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Introduction

Transfusion-transmitted virus (TTV) was identified in 1997 in Japan in a patient with acute post-transfusion non-A-to-G hepatitis.[1] The TTV is an unenveloped and circular DNA virus. TTV possesses a single-stranded DNA genome and

comprises 3537–3853 nucleotides. TTV is similar to the Circoviridae and possesses three open reading frames.[2]

Sequence analysis confirms that the TTV genome contains a high degree of genetic variability and can be classified into at least six major genotypes (Groups 1–6) and several subtypes (1a, 1b, 2a and 2b).[3]

Its frequency varies in different parts of the world. TTV is believed to be hepatotropic as its viral levels are observed to be higher in the liver than in the serum of infected patients. TTV has also been identified within hepatocytes, and was shown to replicate by *in situ* hybridization and polymerase chain reaction (PCR); however, only minor morphologic changes have been seen in cells with positive hybridization signals.[4]

TTV is predominantly transmitted by blood transfusion; hence, its name. Being non-eveloped, TTV is shed via the bile into feces of infected individuals and therefore the fecal–oral route of transmission is possible. The dual mode of transmission of TTV may enhance its deep, wide penetration into the general population. The presence of TTV in many body fluids enhances its ability to be transmitted by the maternal, sexual and respiratory routes.[5]

Chronic kidney disease (CKD) is the condition with irreversible deterioration of renal function leading to derangement and insufficiency of renal excretory and regulatory function.[6] Maintenance hemodialysis (MHD) is employed for sustaining life when CKD patients reach end-stage renal failure (ESRD). Although the benefits of this therapy are unquestioned, many complications have been associated with MHD. The dialysis setting has been recognized as a high-risk environment for the transmission of blood-borne infections to both patients and health care workers. There is a high risk of indirect and direct transmission of infectious agents in MHD units as a vascular access is needed on a regular basis. This results in an increased potential for acquiring nosocomial infections, especially blood borne, via equipment, environmental surfaces or the hands or gloves of any personnel, which become contaminated by potentially infectious blood or other body fluids.[7]

The prevalence of TTV in patients undergoing MHD units varies widely, and various demographic, virologic or clinical features can explain these differences. Blood transfusion requirement and nosocomial transmission of TTV within dialysis units seem to be important in the diffusion of TTV in the MHD setting; however, other routes of TTV acquisition may also play a role.[8]

Objectives

The objectives of this work were to detect the prevalence of TTV infection in HD patients, to study the predictors of occurrence of infection and to determine the most frequent TTV genotype.

Subjects and Methods

A case–control study was conducted at the Arar Central Hospital, Arar City, in the northern border area of Saudi Arabia, from October 2013 to March 2014. Sixty-three (63) patients who had been on MHD therapy for a period more than one year and aged more than 18 years at the Nephrology Center of Arar Central Hospital comprised the study group (Group 1). One hundred age-matched, apparently healthy individuals from the hospital personnel, undergraduates and medical and nursing staff were included as the control group (Group 2). The study was approved by the Research Ethics Committee in the Arar Faculty of Medicine. Informed consent was obtained before participation in this study.

All patients underwent full medical history taking, history of blood transfusion and clinical examination.

About 5 mL of venous blood was collected by sterile venipuncture and allowed to clot naturally; sera were separated and divided into two Eppendorf tubes, one tube sent for biochemical and virological investigations and the second kept frozen at –20°C till use.

Laboratory tests

The biochemical investigation performed were alanine aminotransferase (ALT) and aspartate aminotransferase (AST) using colorimetric methods.[9]

The virological investigations performed were serum hepatitis B surface antigen (HBsAg) and antibody to hepatitis C virus (anti-HCV), which were tested with commercially available enzyme immunoassays (Abbott Laboratories, North Chicago, IL, USA) according to the manuscript.

TTV detection by PCR.

1. Genomic extraction of DNA: Genomic DNA was extracted from the serum using the automated QIAamp DNA Mini Kit (Qiagen, GmbH, Deutschland) according to the manufacturer's instructions for automatic extraction in a QIAcube extractor (Qiagen, GmbH).
2. Amplification of TTV DNA by semi-nested PCR with TTV-specific primers derived from two conserved regions according to the published sequences.[10]

The first round of PCR was performed using primer NG059 (sense primer 5′-ACAGACAGAGGAGAAGGCAACATG-3′) and primer NG063 (antisense primer 5′-CTGGCATTTTACCATTTCCAAAGTT-3′) (Biosearch Technologies, Petaluma, CA, USA). PCR was carried out for 35 cycles consisting of denaturation at 94°C for 45 s, annealing at 58°C for 45 s and extension at 72°C for 45 s, with initial denaturation 1 cycle of 94°C for 5 min and an additional final cycle of extension for 5 min at 72°C using a rapid cycler PCR (G-Storm Thermal cycler, England) and DreamTaq Green PCR Master Mix (2X) (Thermo Scientific, Erlangen, Germany). The second round was performed with another sense primer NG061 (5′-GGCAACATGTTATGGATAGACTGG-3′) and the same antisense primer NG063 using 10 µL of the first round PCR. Amplification was obtained by the same method of first round PCR.

Amplification products 271 bp were visualized on an ethidium bromide-stained 2% agarose gel (Figure 1).

TTV genotyping using restriction fragment length polymorphism analysis (RFLP)

Restriction digestions were carried out with 10 µL of the second round PCR products for 30 min after adjustment with 10 U enzyme reaction buffers according to the manufacturer's instructions. Reactions were carried out with 10 U of NdeI and PstI (Fermentas, Waltham, MA, USA) at 37°C. The digested PCR products were electrophoresed on 2% agarose gels and stained with ethidium bromide. The RFLP pattern was then evaluated under ultraviolet light (Figure 2).

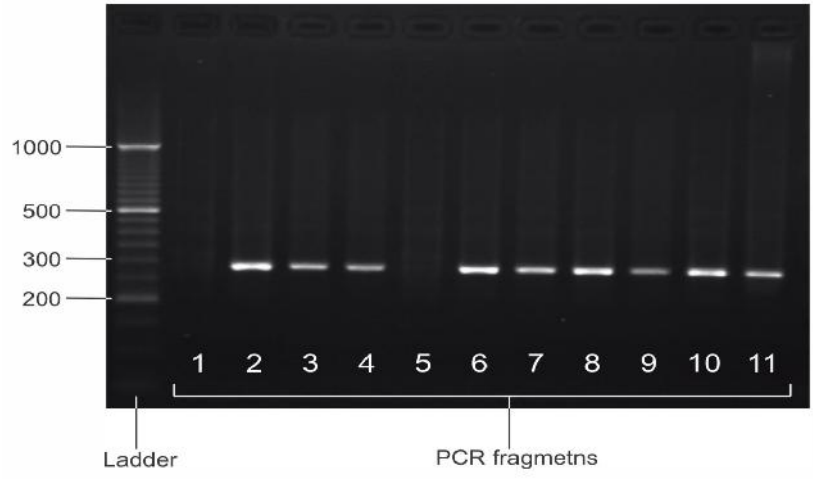

Figure 1. Ethidium bromide-stained gel electrophoresis of the TTV-PCR product showing positive (lanes 2, 3, 4, 6, 7, 8, 9 and 11) and negative (lanes 1 and 5) signals.

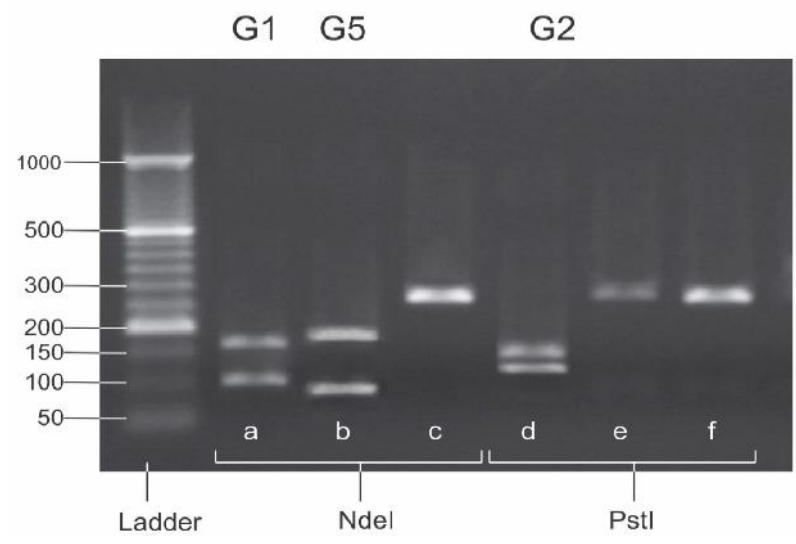

Figure 2. Identification of restriction patterns obtained by restriction endonuclease digestion. Lanes a, b, c: RFLP pattern after NdeI digestion of the ORF1 PCR products. Digestion of G1 with NdeI resulted in 169- and 102-bp fragments (lane a) and digestion of G5 resulted in 183- and 88-bp fragments (lane b). Lanes d, e, f: RFLP pattern after PstI digestion of the ORF1 PCR products. Digestion of G2 with PstI resulted in 147- and 124-bp fragments (lane d). TTV isolates, which were digested by neither NdeI nor PstI, belonged to G4 or G6.

Statistical analysis

The collected data were tabulated and analyzed using SPSS version 20 software. Quantitative data were expressed as mean and standard deviation; qualitative data were expressed as frequencies and percentages. student's *t*-test was used to compare the between mean of two groups, the Chi square "X^2" test was used to compare between categorical data and regression analysis was used to detect predictors of occurrence of TTV infection. P <0.05 was considered to be significant.

Results

Table 1 shows the age and sex of both groups. The mean age of the hemodialysis (HD) group was 42.2 ± 11.7 and that of the control group was 39.2 ± 9.7 and the male/female ratios were 63.5%/36.5% and 53%/47% in group 1 and group 2, respectively. The difference in both variables was statistically non-significant.

Table 2 demonstrates that the prevalence of TTV infection in group 1 was 42.9% while that in group 2 was 19%, and the difference between the two study groups was statistically significant.

Table 1. Cases and control as regards age and sex.

	Group 1 (n = 63)	**Group 2 (n = 100)**	***P*-value**
Age	42.2 ± 11.7	39.2 ± 9.7	>0.05
Sex Male Female	 40 (63.5%) 23 (36.5%)	 53 (53%) 47 (47%)	>0.05

Table 2. Prevalence of TT virus among cases and controls.

	Group 1 (n = 63)	**Group 2 (n = 100)**	***P*-value**
TTV +ve	27 (42.9%)	19 (19%)	<0.001
TTV –ve	36 (57.1%)	81 (81%)	

Table 3. Regression analysis for detection of predictor of occurrence of TTV infection.

	B	95% CI for B*		*P*-value
		Lower	Upper	
Age	1.04	0.98	1.1	>0.05
History of blood transfusion	14.71	2.69	80.32	<0.01
Duration of hemodialysis	0.97	0.92	1.03	>0.05
HBV	0.42	0.07	2.56	>0.05
HCV	0.19	0.03	1.23	>0.05
ALT	1.01	0.96	1.07	>0.05
AST	1.02	0.97	1.07	>0.05

*CI: Confidence interval.

Regression analysis shows that history of blood transfusion was the only significant predictor of occurrence of TTV infection in HD patients (P <0.01), while age, duration of HD, HBV and HCV infection, elevated ALT and AST were not significant predictors for TTV infection (Table 3).

The frequencies of TTV genotypes in the positive cases of group 1 were 70.4%, 7.4%, 0%, 14.8% and 7.4% for G1, G2, G3, G4 or G6 and G5, respectively, whereas the frequencies in the positive cases of group 2 were 42.1%, 10.5%, 5.3%, 15.8% and 26.3%, respectively (Table 4).

Discussion

The TTV is a DNA virus that was first identified in patients with non-A to-G hepatitis following blood transfusion and was named TTV. TTV can be transmitted by blood products and through common parenteral routes. Patients on HD are considered to be at risk of infection by various blood-borne viruses, including TTV.[11]

The frequency of TTV infection among patients on MHD varies widely. The geographical distribution, the methods used for TTV DNA testing, the size of the study group and the presence of various demographic, virological or clinical features of dialysis patients contribute to the differences.[8]

The aim of our study was to detect TTV-DNA, its genotype in HD patients and to study the predictors for occurrence of infection.

The prevalence of TTV among HD patients ranged from 2.5% to 53% in various studies: 2.5% in the study by Halfon et al in France,[12] 53% in the study by Forns et al in Spain,[13] 30% and 51.3% in studies by Oguchi and Utsunomiy in Japan,[14,15] 9.3% in the study by Kheradpezhouh et al in Tabriz, northwestern Iran,[16] 64.8% in the study by Abou-Donia et al in Egypt[5] and 16.7% in the study by Ataei et al in Iran.[4]

In our study, the prevalence of TTV in HD patients was statistically significantly higher (42.9%) compared with (19%) that in the control group (P <0.001). This was in agreement with Chan et al,[17] who found that TTV was 61% among patients on HD compared with 15.6% in healthy donors15.6%. Our work also agreed with Martinez et al,[18] who revealed statistical significance of TTV in HD patients (16%) than that among the healthy population (2%) (P <0.001) and with Rivanera et al,[19] who reported that the prevalence of TTV-DNA in dialysis patients (41.7%) was significantly higher

Table 4. Frequency distribution of TTV genotype in positive cases in both groups.

	Group 1 (n = 27)		Group 2 (n = 19)	
	No.	%	No.	%
G1	19	70.4	8	42.1
G2	2	7.4	2	10.5
G3	0	0	1	5.3
G4 or G6	4	14.8	3	15.8
G5	2	7.4	5	26.3

than that in the healthy population (10.7%) (P <0.001). However, in contrast to our study, Barril et al[20] found that TTV was similar in both HD patients and healthy blood donors (22.7% and 20%, respectively).

Our result is not in agreement with Kanamoto et al,[21] who found that TTV was less prevalent in HD patients than that in the general population in Japan and that the virus was more often eliminated in HD patients than in the general population during the three-year observation period, possibly because of the effect of the HD process.

The prevalence of TTV in healthy blood donors ranged from 1% to 40% in early studies.[22] As more inclusive primers were used to detect the different genotypes, the reported prevalence among blood donors increased dramatically, approaching 100% in some studies.[23] Vasilyev et al in Russia[24] reported that the prevalence of TTV in the healthy blood donors was 94%. However, in our study, the prevalence of TTV in healthy blood donors was 8.3%. The fact that TTV is also detectable in the healthy population confirms the role of non-parenteral routes of transmission.

In studying the predictors of occurrence of TTV infection in HD patients, the only significant predictor was history of blood transfusion, and this is supported by many other studies.[5,17,25,26,27] This is in contrast to Hsu et al,[11] Kheradpezhouh et al[16] and Martinez et al,[18] who revealed that there was no significant association between TTV positivity and history of blood transfusion. These data suggest that TTV may be transmissible not only by blood but also by the non-parenteral route. This observation has been supported by the fact that TTV DNA is usually detected by PCR in serum and other biological fluids.[28]

In addition, our study provided evidence that age of patients, duration of HD, hepatitis B and C infection and AST and ALT levels were not significant predictors of TTV positivity in HD patients. This result coincides with that reported by many other studies.[5,11,18,26] This is also consistent with the results of Asim et al and Irshad et al,[28,29] who found no biochemical evidence of a link between liver disease and TTV infection in the TTV-DNA-positive HD patients. In contrast to our study, Itoh et al[30] reported a higher prevalence of TTV in blood donors with elevated ALT levels (32%) than in those with normal levels (16%). Also, Kheradpezhouh et al[16] found that TTV-positive patients were significantly younger than TTV-negative patients.

TTV is characterized by an unusually high degree of sequence variability compared with other DNA viruses, and several distinct TTV genotypes have been described.[10,21] The sequence analysis of the TTV-DNA-positive samples revealed that genotype 1 was the most frequent genotype in the disease group as well as among the healthy controls.

Our result was in agreement with Asim et al and Irshad et al,[28,29] who stated that TTV DNA genotype 1 (G1) was found to be the main genotype in HD patients.

The prevalence of TTV in HD patients is significantly higher than that in healthy individuals. History of blood transfusion was the only significant predictor of TTV positivity in HD patients. Genotype 1 was the most predominant type in HD and healthy individuals.

Recommendations

It seems necessary to take serious measures to reduce the risk of TTV transmission to HD patients. Further studies on TTV in peritoneal dialysis patients and transplant patients are needed.

Conflict of interest: None

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