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Full Length Research Paper

The prevalence of transfusion-transmitted virus (TTV) infection in patients with chronic hepatitis B and C in southwest of Iran

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Transfusion-transmitted virus (TTV) is an unenveloped circular single-stranded DNA virus with a diameter of 30 to 32 nm that was first described in 1997 in Japan. TTV was detected in various populations without proven pathology, including blood donors and in patients with chronic hepatitis B virus (HBV) and hepatitis C virus (HCV). The aim of this study was to determine the prevalence of TTV DNA in patients with chronic hepatitis B and C in southwest Iran. Viral TTV-DNA was studied in 442 samples (202 with HBV, 138 with HCV and 102 controls) collected from southwest of Iran. All the extracted serum DNA was amplified by *TTV ORF1* gene specific primers using the semi nested polymerase chain reaction (PCR) technique. TTV DNA was detected in the serum of 8.9 and 10.8% patients with chronic hepatitis B and C, respectively. The prevalence of TTV-DNA in the serum of the 102 controls was 2.9%. The results showed significant relationship of TTV with HBV and HCV in patients from T test examination (P < 0.01). The prevalence of TTV-DNA in Iranian hepatitis B and C patients was rather high when compared with those of other countries. To control and prevent the distribution of TT-virus, examination of the blood and blood products seems necessary.

Key words: Transfusion-transmitted virus (TTV), hepatitis C virus (HCV), hepatitis B virus (*HBV*), *ORF1* gene, semi nested polymerase chain reaction (PCR), Iran.

INTRODUCTION

Transfusion-transmitted virus (*TTV*) was isolated from the serum of a Japanese patient with fulminant hepatitis and chronic liver disease of unknown etiology (Hsieh et al., 1999). TTV, like parvovirus, does not have an envelope. Its genome consists of a single-stranded, linear DNA molecule of about 3.818 to 3.853 nucleotides in length (Okamoto et al., 1998). TTV is a member of the *Circoviridae* family and *Anellovirus* genus, and has not been cultured *in vitro* and its pathogenic potential is still not clear (Abe et al., 1999). TTV DNA has been detected in the blood of newborns, cord blood, semen, saliva, cervical swabs and in amniotic fluid (Fornai et al., 2001; Okamoto et al., 1998; Tanaka et al., 2001). The TTV chronically infects healthy individuals of all ages in different populations of the world (Alfaresi et al., 2006). TTV is transmitted parenterally and typically by transfusion of blood and blood products, and is shed via the bile into the feces of infected individuals for possible fecal-oral transmission (Muljono et al., 2001). TTV is found in the plasma and peripheral blood mononuclear cells, different body fluids and secretions such as stools, saliva, semen, vaginal fluid, breast milk and tears (Inami et al., 2000; Kheradpezhouh et al., 2007). TTV also has been found in other organs including kidneys, prostate, mammary glands, brain and bone marrow cells (BMCs) (Irshad et al., 2006; Wagner et al., 2006).

Hepatitis B and C viruses (HBV and HCV) cause transient and chronic infections of the liver, which may progress to cirrhosis and eventually to hepatocellular carcinoma (HCC). Coinfection of TTV and HBV or TTV and HCV is common, because these viruses share the same transmission routes such as blood transfusion

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Abbreviations: TTV, Transfusion-transmitted virus (Torque Teno virus); **HBV,** hepatitis B virus; **HCV**, hepatitis C virus.

(Garcia et al., 2003; Polz et al., 2008). Prevalence of TTV ranges from 1.9 to 37%, respectively, in the general population or in healthy voluntary blood donors in different countries (Huang et al., 2000). Coinfection of HBV infected patients with TTV differs from 8 to 35%. The Data of HCV and TTV coinfection are similar to the earlier which is within the range of 8 and 42% (Schreter et al., 2004). According to the report in 2007, the seroprevalence of TTV was 9.3% in Iranian hemodialysis patients (Kheradpezhouh et al., 2007).

TTV was originally found in humans; however, recent studies showed that TTV can also be identified in serum specimens obtained from domesticated farm animals and from non-human primates. One study demonstrated frequent TTV infection of domestic animals such as cows, pigs, sheep and chickens (Simmonds et al., 1999). However, it is unknown how these species acquire TTV infection. There are some reports showing high prevalence of TTV infection in captured chimpanzees and crabs eating macaques (Inami et al., 2000). These findings suggest that TTV is widespread among wild chimpanzees living in West Africa (Irshad et al., 2006).

Many studies have shown that TTV is not the causative agent of chronic liver disease of unknown etiology and neither does it affect the degree of liver damage when present as a coinfection with HBV or HCV (Irshad et al., 2006). Yet, no significant difference between TTV infected and non-infected patients were found for the demographic data, assumed source of infection, biochemical abnormalities or severity of liver histology (Tangkijvanich et al., 1999). Thus, regarding etiology and progression towards serious chronic liver disease, its contribution seems to be minor if not all together non-existent. Concerning antiviral therapy, there are no data or treatment for patients who are infected with TTV alone since the role of TTV as a cause of chronic hepatitis is yet to be determined (Irshad et al., 2006).

The aim of this study was to determine the prevalence of TTV in patients with chronic HBV and HCV in the southwest of Iran via the tracing of the *ORF1* gene of this virus by using a semi nested polymerase chain reaction (PCR) method.

MATERIALS AND METHODS

Sampling

After agreement with the private and governmental clinical pathologic laboratories and clinical centers in southwest of Iran, 340 serum samples were collected from the patients with HBV and HCV (202 and 138 HBV DNA and HCV RNA positive, respectively). Then, the samples of 102 controls (without hepatitis B or C) with the permission of the patients (during 2010) were then transferred to the Biotechnology Research Center in ice and stored at -70°C.

Population study consisted of 102 controls without hepatitis B or C (55 men and 47 women; median age: 43.12, range: 25 to 68 years) and 340 patients (189 men and 151 women; median age: 47.66 years, range: 24 to 66 years) with chronic HBV or HCV.

Nucleic acid extraction

DNA was extracted by DNA extraction kit (QIAGEN Ltd., Crawley, UK) according to the manufacturer's procedure. The yield of DNA was quantified after electrophoresis in 1% agarose gel containing 0.5 µg/ml of ethidium bromide.

Determination of *TTV*-DNA by semi nested PCR

TTV DNA was determined by semi nested PCR with the use of 3 primers described by Okamoto et al. (2001) for *ORF1* gene (accession number: AF151683). The three primers were a forward primer for *ORF1* gene which was *TTV*-F: 5´- ACAGACAGAGGA GAAGGCAACATG -3´, reverse primer for *ORF1* gene which was *TTV-R*: 5´- CTGGCATTTTACCATTTCCAAAGTT -3´ and another forward primer for the gene was *TTV*-FF: 5´- GGCAACATGTTATG GATAGACTGG -3´ (Okamoto et al., 2001).

Gene amplification

PCR was performed in a 50 µl total volume containing 1 µg of template DNA, 1 µM of each primers, 2 mM $Mgcl_2$, 200 µM dNTP, 5 µl of 10X PCR buffer and 1 unit of Taq DNA polymerase (Roche applied science). The following conditions of PCR were used for gene amplification for the first round: initial denaturation at 95°C for 5 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 58°C for 1 min and extension at 72°C for 1 min. The program was followed by a final extension at 72°C for 6 min. 2 µl from the first round amplicon was used as a template for the second round PCR. The second round PCR was performed with TTV-FF and TTV-R oligonucleotide primers for 25 cycles with the same condition. The PCR product was analyzed by electrophoresis in 1% agarose gel in 1X TBE buffer and was visualized by ethidium bromide staining on UV transilluminator.

RESULTS

The analysis of PCR products of *ORF1* gene of TTV on agarose gel revealed a 271 bp fragment (Figure 1). In this study, all the collected samples were examined for the presence of TTV DNA. For further characterization, clinical background including mean age, sex and transfusion history of TTV-PCR positive and negative patients were evaluated.

The prevalence of TTV in the controls (without hepatitis B or C) and patients with chronic HBV and HCV was 2.9, 8.9 and 10.8%, respectively, and these results showed the significant relationship between TTV and the patients that had chronic HBV and HCV with 99% confidence level by T test ($P < 0.01$). Table 1 shows the prevalence of TTV-DNA in the serum samples.

DISCUSSION

TTV was first reported in Japan in 1997 by Nishizawa in patients with fulminant hepatitis and chronic liver disease of unknown etiology (Irshad et al., 2006). The association between TTV infection and hepatitis is controversial (Mushahwar, 2000; Okamoto et al., 2000). This virus was

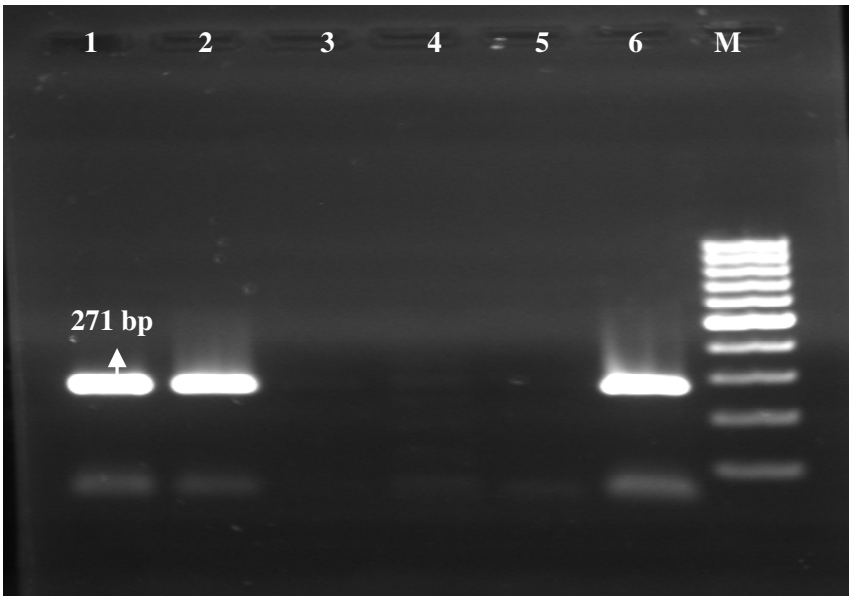

Figure 1. Identification of TT virus by semi nested PCR amplification of the *ORF1* gene. Lanes 1 and 2 are positive samples of TTV, lanes 3 and 4 are negative samples, lanes 5 and 6 are negative and positive controls, respectively. M, 100 bp DNA ladder (Fermentas, Germany).

Table 1. Prevalence of TTV-DNA in the serum samples of 340 hepatitis patients and 102 controls (without hepatitis B or C).

Sample	Number	Positive (%)	Negative (%)
HBV-positive	202	18 (8.9)	184 (91.1)
HCV-positive	138	15 (10.8)	123 (89.2)
Controls (without hepatitis B or C)	102	3 (2.9)	99 (97.1)
Total	442	36 (8.1)	406 (91.9)

initially identified in a large number of patients with acute and chronic hepatitis patients in most countries (Irshad et al., 2006; Zandieh et al., 2005). Concomitant infection with TTV and either HBV or HCV is common. However, the effect of TTV infection in patients with chronic HBV or HCV infection is unknown (Arankalle et al., 2007).

According to the result of this study, the prevalence of TTV in patients with chronic HBV and HCV in the south-west of Iran was 8.9 and 10.8%, respectively. TTV-DNA levels in the liver tissue were equal to or 10 to 100 times higher than those in the serum, suggesting that this virus is replicated in the liver (Irshad et al., 2006). The prevalence of TTV infection caused by blood transfusion also differs depending on the country or area. Using the PCR, epidemiological studies have indicated a worldwide distribution of this virus, with prevalence surveys in the general population of 12 to 19% in Japan (Okamoto et al., 1998; Nagano et al., 1999), 36% in Thailand (Tanaka et al., 1998), 2 to 10% in European countries (Simmonds et al., 1998; Naoumov et al., 1998) and 1% in the USA (Irshad et al., 2006). The prevalence of TTV in Iranian patients with chronic HBV or HCV was the same with the prevalence of this virus in the European countries and was different from those of Japan and Thailand.

The prevalence of TTV DNA in the western India varied from 6.7% (5 of 75) in chronic hepatitis patients, 24.4% (10 of 41) in hemophiliacs to 7.4% (4 of 54) in voluntary blood donors and this result was the same with the prevalence in this study (Arankalle et al., 20007). The prevalence of TTV-DNA in thalassemic patients and blood donors in Iran was 57.2 and 20%, respectively (Zandieh et al., 2005).

Recent studies suggested that TTV infection is a relatively common virus infection throughout the world in different places and different racial groups (Nishizawa et al., 1997; Okamoto et al., 1998). According to this finding, TTV is highly associated with HBV and HCV infections and the region of this study is at risk for this virus.

Since TTV was discovered a few years ago, many studies have been done to assess whether it causes liver

disease; however, there is still a poor understanding of its molecular properties and pathogenic potential. So, the results of this research confirmed the results of previous studies. It was shown that TTV infection is acquired in many patients with chronic HCV and HBV in Iran. On the other hand, many researches have shown that the prevalence of TTV DNA was higher in patients that had received several blood transfusions or blood products. So, examination of blood samples for TTV seems necessary.

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