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The Prevalence of Transfusion-Transmitted Virus (*TTV*) Infection in Iranian Patients with Chronic Hepatitis B

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

***Keywords*—**Transfusion-transmitted virus (*TTV*), Hepatitis C virus (*HCV*), Hepatitis B virus (*HBV*), *ORF1* gene, Semi nested PCR, Iran.

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I. Introduction

TRANSFUSION-transmitted virus (*TTV*) was isolated from the serum of a Japanese patient with fulminant hepatitis and chronic liver disease of unknown etiology [1]. *TTV*, like parvovirus, does not have an envelope. Its genome consists of a single-stranded, linear DNA molecule about 3.818-3.853 nucleotides in length [2]. *TTV* is a member of *Circoviridae* family and *Anellovirus* genus, and has not been cultured in vitro and its pathogenic potential is still not clear [3]. *TTV* DNA has been detected in blood of newborns, in cord blood, semen, saliva, cervical swabs and in amniotic fluid [2], [4], [5]. The *TTV* chronically infects healthy individuals of all ages in different populations of the world [6]. *TTV* is transmitted parenterally, 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 [7]. *TTV* is found in plasma and peripheral blood mononuclear cells, different body fluids and secretions such as stools, saliva, semen, vaginal fluid, breast milk and tears [8], [9]. *TTV* also has been found in other organs including kidneys, prostate, mammary glands, brain and bone marrow cells (BMCs) [10], [11].

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 commonly occurring, because these viruses share the same transmission routes such as blood transfusion [12], [13]. Prevalence of *TTV* ranges from 1.9% to 37%, respectively, in general population or in healthy voluntary blood donors in different countries [14]. Coinfection of *HBV* infected patients with *TTV* differs from 8% to 35%. Data about *HCV* and *TTV* coinfection are similar to above within the range from 8% to 42% [15]. According to the report in 2007, the seroprevalence of *TTV* was 9.3% in Iranian hemodialysis patients [9].

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 has demonstrated frequent *TTV* infection of domestic animals such as cows, pigs, sheep and chickens [16]. 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 [17]. These findings suggest that *TTV* is widespread among wild chimpanzees living in West Africa [18].

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* [18]. Yet, no significant differences between *TTV* infected and non-infected patients were found as to demographic data, assumed source of infection, biochemical abnormalities, or severity of liver histology [19]. 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 of patients who are infected with *TTV* alone since the role of *TTV* as a cause of chronic hepatitis has yet to be determined [18].

The aim of this study was to determine the prevalence of *TTV* in patients with chronic *HBV* and *HCV* in the west south

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of Iran via tracing *ORF1* gene of this virus by using a semi nested PCR method.

II. Materials and Methods

Sampling

After agreement with private and governmental clinical pathologic laboratories and clinical centers in west south of Iran 340 serum samples were collected from the patients with *HBV* and *HCV* (202 and 138 *HBV* DNA and *HCV* RNA positive, respectively). And, 102 controls (without hepatitis B or C) with the permission of the patients during 2010, then transformed the samples to the biotechnology research center in ice and stored at -70ºC.Population study consisted of 102 controls without hepatitis B or C (55 men and 47 women; median age: 43.12 range: 25 and 68 years) and 340 patients (189 men and 151 women; median age: 47.66 years; range: 24 and 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 for *ORF1* gene (accession number: AF151683). The three primers are a forward primer for *ORF1* gene was *TTV*-F: 5´-ACAGACAGAGGAGAAGGCAACATG -3´, and reverse primer for *ORF1* gene was *TTV-R*: 5´-CTGGCATTTTACCATTTCCAAAGTT -3´, and another forward primer for this gene was *TTV*-FF: 5´-GGCAACATGTTATGGATAGACTGG -3´ [20].

Gene amplification

PCR was performed in a 50µl total volume containing 1µg of template DNA, 1µM of each primers, 2mM $Mgcl_2$, 200µM dNTP, 5µl of 10X PCR buffer and 1 unit of Taq DNA polymerase (Roche applied science). The following conditions for first round of PCR, were used for gene amplification: initial denaturation at 95ºC for 5 min, followed by 30 cycles of denaturation at 94ºC for 1 min, annealing at 58ºC for 1 min and extension at 72ºC for 1 min. The program was followed by a final extension at 72ºC for 6 min. Two 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 visualized by ethidium bromide staining on UV transilluminator.

III. Results

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

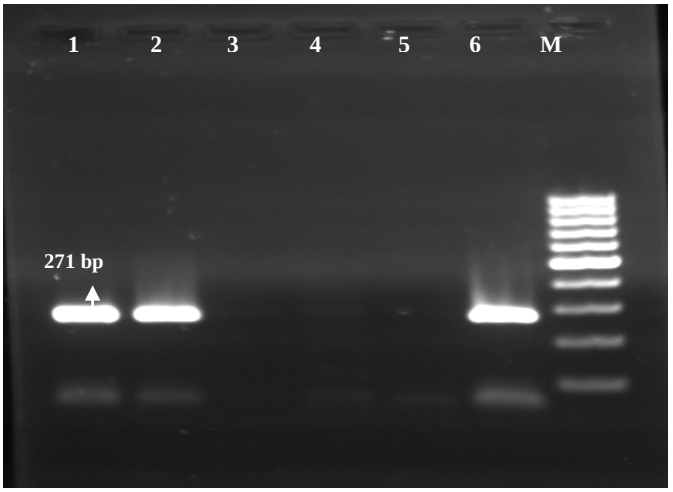


Fig. 1 Identification of *TT virus* by semi nested PCR amplification of the *ORF1* gene. Lanes 1and 2 are positive samples of *TTV*. Lanes 3 and 4 are negative samples. Lanes 5 and 6: negative and positive controls respectively. M: 100 bp DNA ladder (Fermentas, Germany).

The prevalence of *TTV* in controls (without hepatitis B or C) and patients with chronic *HBV* and *HCV* was 2.9, 8.9 and 10.8 percent respectively, and these results showed the significant relationship between *TTV* and patients that have chronic *HBV* and *HCV* with 99% confidence level by T test ($P<0.01$).There is statistical differ between *TTV*-DNA positive and negative with age and transfusion history by T test with 99% confidence level ($P<0.01$), but was not differ between *TTV*-DNA positive and negative and sex. Table I showed the prevalence of *TTV*-DNA in the serum samples.

TABLE I
PREVALENCE OF *TTV*-DNA IN YHE SERUM SAMPLES OF 340 HEPATITIS PATIENTS AND 102 CONTROLS

Samples	Number	Positive (Percent)	Negative (Percent)
***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%)

IV. Discussion

TTV was first reported in Japan in 1997 by T. Nishizawa in patients with fulminant hepatitis and chronic liver disease of unknown etiology [18]. The association between *TTV* infection and hepatitis is controversial [21], [22]. This virus was initially identified in a large number of patients with acute and chronic hepatitis patients in most countries [18], [23]. Concomitant infection with *TTV* and either *HBV* or *HCV* is

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common. However, the effect of *TTV* infection in patients with chronic *HBV* or *HCV* infection is unknown [24].

According to the result of this study the prevalence of *TTV* in patients with chronic *HBV* and *HCV* was 8.9 and 10.8 percent respectively. *TTV*-DNA levels in liver tissue were equal to or 10-100 times higher than those in serum, suggesting that this virus replicated in the liver [18]. The prevalence of *TTV* infection caused by blood transfusion also differs depending on the country or area. Using the polymerase chain reaction (PCR), epidemiological studies have indicated a worldwide distribution of this virus, with prevalence surveys in the general population reporting values of 12% to 19% in Japan [2], [25], 36% in Thailand [26], 2% to 10% in European countries [16], [27] and 1% in the USA [18]. The prevalence of *TTV* in Iranian patients with chronic *HBV* or *HCV* was same to the prevalence of this virus in European countries and different from Japan and Thailand. Prevalence of *TTV* DNA in western India was varied from 6.7% (5 of 75) in chronic hepatitis patients, 24.4% (10 of 41) in hemophiliacs and 7.4% (4 of 54) of voluntary blood donors and this result same to prevalence of resent study [23], [24]. The prevalence of *TTV*-DNA in thalassemic patients and blood donors in Iran was 57.2% and 20% respectively [23].Recent studies suggest that *TTV* infection is a relatively common virus infection throughout the world in different places and different racial groups [2], [28]. According to this finding *TTV* have highly associated with *HBV* and *HCV* infections and region of current study is the risk situation for this virus.Since *TTV* was discovered a few years ago, many studies have been done trying 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 confirm the results of previous studies. Since, we have shown that *TTV* infection is acquired in many patients with chronic *HCV* and *HBV* in Iran. On the other hand many of research have shown that prevalence of *TTV* DNA to be higher in patients having received several blood transfusions or blood products. So examination of blood samples to finding *TTV* it seems necessary.

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