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

P. Ghasemi Dehkordi*, A. Doosti, M. R. Hajimirzaei

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 TTV 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.

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...
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 -70°C.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'-ACAGACAGAGGAGGAAGGCAACATG -3’, and reverse primer for ORF1 gene was TTV-R: 5'-CTGGCAATTTACCATTTCCAAGTT -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, 1μl of each primers, 2mM MgCl2, 200μM dNTP, 5μl of 10X PCR buffer and 1 unit of Taq DNA polymerase (Roche applied science). The following conditions were used for first round of PCR, were used for gene amplification: 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. 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.

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>
<thead>
<tr>
<th>Samples</th>
<th>Number</th>
<th>Positive (Percent)</th>
<th>Negative (Percent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBV-Positive</td>
<td>202</td>
<td>18 (8.9%)</td>
<td>184 (91.1%)</td>
</tr>
<tr>
<td>HCV-Positive</td>
<td>138</td>
<td>15 (10.8%)</td>
<td>123 (89.2%)</td>
</tr>
<tr>
<td>Controls (without hepatitis B or C)</td>
<td>102</td>
<td>3 (2.9%)</td>
<td>99 (97.1%)</td>
</tr>
<tr>
<td>Total</td>
<td>442</td>
<td>36 (8.1%)</td>
<td>406 (91.9%)</td>
</tr>
</tbody>
</table>

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...
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 recent study [23], [24]. The prevalence of TTV-DNA in thalassemics 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 HBV and HCV 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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REFERENCES


