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Original Article

Molecular Epidemiology of Torque Teno Virus (TTV) Isolated from in Healthy and Subjects with Chronic Hepatitis B and C in Jahrom City of Iran

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Abstract

Background and Aims: Torque teno virus (TTV) is a DNA virus that isolated from the serum of a Japanese patient without A-G transfusion-transmitted hepatitis and other etiology. TTV is detectable in plasma and peripheral blood mononuclear cells, different body fluids and secretions such as stools, saliva, semen, vaginal fluid. The genome exhibits high diversity that has enabled the determination of several genotypes and subtypes and at least 30 genotypes have been identified. TTV might be seen as a risk factor in acute and chronic hepatitis, but it is not clear. Coinfection of TTV and HBV or TTV and HCV is common, because these viruses share the same transmission routes such as blood transfusion. The aim of this study was to determine the prevalence of TTV in patients with chronic HBV and HCV in the Southern of Iran and evaluate effect of TTV infection on the liver diseases.

Materials and Methods: Serum samples collected from all hepatitis patients and healthy control subjects were included for serological tests for hepatitis B and C viruses. Briefly, DNA was isolated from serum of collected peripheral blood mononuclear cells (PBMC) of patients and carry out Semi Nested PCR Detection for TTV DNA.

Results: These results showed the significant relationship between TTV and the patients that had chronic HBV and HCV ($p<0.01$).

Conclusion: According to the result of this study, the prevalence of TTV in patients with chronic HBV and HCV in the southern of Iran was 50.8 and 66.5 %, respectively. These results were comparable to those reported in previous studies ranging from 20% to 75.7% for hepatitis C and from 40% to 75% for hepatitis B patients.

Keywords: Torque Teno Virus; Coinfection; HBV; HCV

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Introduction

Torque teno virus (TTV) is a DNA virus that was isolated from the serum of a Japanese patient without A-G transfusion-transmitted hepatitis (1, 2). This virus is non-enveloped with single-stranded circular DNA genome of negative polarity, 3.4-3.9 Kb in length, and several open reading frames that is classified to a new genus called Anellovirus, belonging to the Circoviridae family (3, 4). Detection of TTV derived from

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clinical samples of infected individuals showed that TTV is detectable in plasma and peripheral blood mononuclear cells, different body fluids and secretions such as stools, saliva, semen, vaginal fluid (5, 6). Despite other DNA viruses, TTV exhibits drastic wide sequence diversity (2). The genome exhibits high diversity that has enabled the determination of several genotypes and subtypes and at least 30 genotypes have been identified (7).

Hepatitis B and C viruses (HBV and HCV) cause transient and chronic infections of liver, which may progress to cirrhosis and eventually to hepatocellular carcinoma (HCC). TTV might be seen as a risk factor in acute and chronic hepatitis, but it is not clear (8). Several studies showed the presence of TTV in patients infected with HCV which suggests that TTV titer is correlated with severity of carcinoma attributed to HCV and therefore it can be used as a prognostic predictor for the outcome of chronic HCV infection (9, 10). Coinfection of TTV and HBV or TTV and HCV is common, because these viruses share the same transmission routes such as blood transfusion. Further studies showed that accumulated TTV DNA titers correlate with the level of aminotransferase in the patients. Several clinical findings together with detected TTV DNA in 47 % of patients with fulminant hepatitis and in 46 % of patients with chronic liver disease of unknown etiology support the proposal that TTV may deserve partly as a possible agent for acute and chronic liver diseases of unknown etiology (11). Coinfection of HBV and HCV infected patients with TTV differs from 8 to 35% and within the range of 8 and 42% respectively (12). Also TTV is distributed in more than 50 % of normal human population throughout the world (2).

Diversity of this virus in comparison with other DNA viruses is very high and more than 30 genotypes until now in genotypic studies are identified (7, 13). In many genotyping studies used sequences of UTR region reported that genotypes 1, 2, and 3 are highly prevalent worldwide (14, 15). The UTR region is comparatively well conserved and contains several regions with an identity greater than 90 % indicating that high variation is not tolerated in these regions therefore it seems that genotyping based on UTR sequence is more accurate and reliable (16-18). In recent years, several studies performed to determine the prevalence, modes of transmission, and clinical relevance of TT virus. However, no data are available in regards to circulating TTV genotypes in patients from Jahrom city, Iran. The aim of this study was to determine the prevalence of TTV in patients with chronic HBV and HCV in the Southern of Iran and evaluate effect of TTV infection on the liver diseases.

Methods

Study Population

After agreement with Honary Medical Clinic Centre in Jahrom, a town in south part of Iran, between September 2012 and February 2013 and having signed informed consents, a total of 159 serum samples were collected from the patients with HCV (102 HCV RNA and Anti-HCV and 57 HBV hepatitis B surface antigens (HBs Ag) positive). The mean age of the patients was 41.03±12.12 years (range 21-68 years). Males comprised the majority 119 (74.8%) and most of the patients were over 40 years old (55.9%). Of course the status of these patients was classified into chronic hepatitis and liver cirrhosis (LC). Also, Population study consisted of 102 controls without hepatitis B or C that transferred to the Research Center in ice and stored at -70°C. They were 39 men and 61 women ranging in age from 21 to 72 years of age (mean±SD: 42.6±14.3 years). The healthy controls (n=100) were drawn from healthy men and women voluntary blood donors with normal liver function test (LFT) profiles and serologically negative for HBV and HCV.

Serology and Isolation of Viral Nucleic Acid

Serum samples collected from all hepatitis patients and healthy control subjects were included for serological tests for hepatitis B and C viruses viz., HBsAg using Eliscaan micro ELISA strips (Ranbaxy Diagnostics, England), Anti-HCV by Innotest HCV AB III

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(Innogenetics NV, Ghent, Belgium). Serum Alanine Aminotransferase (ALT) levels were determined using commercially available Hitachi-7600 series analyzer. Briefly, DNA was isolated from 140 µL serum of collected peripheral blood mononuclear cells (PBMC) of patients by using QIAamp Viral DNA Mini Kit (QIAGEN) based on manufacturer's instruction then preserved at -70°C until analysis.

Semi Nested PCR Detection for TTV DNA

The primer pair sequences were performed in previous studies (13). The first rounds of TTV nested PCR reactions for 5′-untranslated region (5′-UTR) 5′-UTR region contained 5 µL of template DNA, 0.3 µL (10pM stock) of each amplification primer, respectively, 0.5 µL of dNTP (10 mM stock), 2.5 µL of Taq DNA polymerase (Fermentas GmbH, Germany), 0.5 mM of MgCl2, and 2.5 µL of 10×buffer (500 mM of KCl and Tris-HCl, pH 8.4). PCR amplifications were performed as follows: initial denaturation at 95°C, 5 min and 35 cycle's of 40 sec each at 94°C, 45 sec at 60°C, and 50 sec at 72°C, with a final 7 min extension at 72°C. The PCR products were separated by electrophoresis in 2% agarose gels and visualized after staining by ethidium bromide. The final cycling program was employed as follows: initial denaturation at 95°C, 5 min and 35 cycles of 50 sec each at 94°C, 40 sec at 65°C, and 40 sec at 72°C, with a final 3 min extension at 72°C. Program used for the second PCR round was designed similar to the first round PCR except for the number of cycles that were reduced to 25 and reaction template was selected equal to 2 µL from the first round product. The amplification products of the second PCR round were 220 bp.

Statistics

Results were expressed as mean±SD. Difference proportions were tested by the Chi-square/Fisher exact test. Mean quantitative values were compared by student t-test between two groups. Differences were considered to be statistically significant at $p<0.05$.

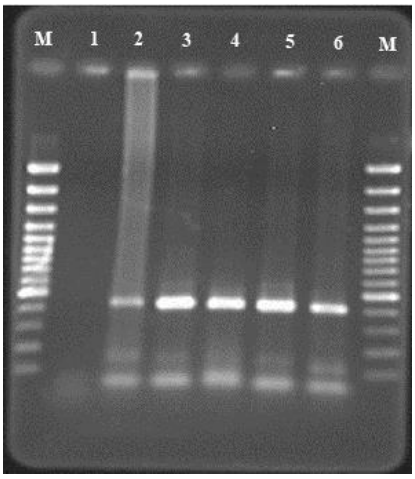

Fig. 1. RT-Nested PCR results on agarose gel (1%) electrophoresis of different samples by outer and inner primer of NP gene: Lanes1: molecular marker (100-bp ladder); lanes 2: CDV Onderstepoort strain; lane 3: Suspected sample; lanes 4: Ultrapure water and 5: Alk strain of measles virus. Numbers on the left are molecular sizes (in base pairs).

Results

Our data shows that 35.8% and those who had CLD were infected with HBV and 64.2% with HCV. Cirrhotic patient formed 10.6% of CLD that all of patients were infected-HCV. TTV-DNA was detected in 68 out of 102 (66.5%) patients with HCV disease and 29 out of 57 (50.8%) patients with HBV disease by 5′-UTR primer based PCR method and also in 18 out of 100 (18%) of healthy individuals. Also TTV-DNA was detected in 100% of the cirrhotic patients. These results showed the significant relationship between TTV and the patients that had chronic HBV and HCV ($p<0.01$). Table 1 shows the prevalence of TTV-DNA in the serum samples. The analysis of PCR products of 5′-UTR region of TTV revealed a 220 bp fragment (Figure 1).

Effect of TTV infection on the liver

The effect of TTV infection (as determined by 5′-UTR positivity) on the liver was investigated by measuring the liver enzyme, ALT values for each patients in all groups positive and negative for TTV DNA (Table 2). Healthy individuals infected with TTV (n=18)

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Table 1. Positivity of TTV in patients with various types of chronic hepatitis and controls.

Groups	No	ALT (mean± SD)	Total TTV positive (%)
Healthy individuals	100	31±84	18 (18)
HCV-infected	102	65±21	68 (66.5)
HBV-infected	57	71±54	29 (50.8)
Total	259	-	149 (57.2)

Table 2. Effect of TTV positivity on the outcome of hepatitis infection and healthy individuals based on ALT levels.

Groups		No	ALT (mean±SD)
Healthy individuals	TTV(+)	18	34±24
	TTV(-)	82	32±71
HCV-infected	TTV(+)	68	65±21
	TTV(-)	34	59±52
HBV-infected	TTV(+)	29	71±54
	TTV(-)	28	67±82
Total	-	259	-

had relatively higher levels of ALT (34±24) than those who were not infected. In the HBV and HCV patients groups, subject's positive for TTV infection had higher levels of ALT than those with single HBV and HCV. All of hepatitis patients who were positive for TTV DNA (n=97) had relatively higher levels of ALT (68±54) than those who were negative for TTV DNA (61±37). Cirrhotic patients had higher levels of ALT (81±52) than those CLD patients. However, all these difference were no statistically significant ($p<0.14$).

Discussion

The purpose of recent study was to survey the rate of infection of TTV in the Jahrom, Southern of Iran. TTV was first originally isolated from a patient with fulminant hepatitis in Japan in 1997 by Nishizawa and also from chronic liver disease of unknown etiology and cryptogenic hepatitis (19). These findings assumed that TTV may associate with hepatitis, although to date a causative effect has not been established (20). TTV has been found to be extremely common in humans with high worldwide prevalence, patients with a broad spectrum of hepatic disorders concomitant with HBV or HCV (19, 21) as well as in healthy people that is very variable, and also in order to clear pathology of TTV infection, further studies should be performed to evaluate prevalence of TTV infection in region patients.

According to the result of this study, the prevalence of TTV in patients with chronic HBV and HCV in the southern of Iran was 50.8 and 66.5%, respectively. These results were comparable to those reported in previous studies ranging from 20% to 75.7% for hepatitis C (22-24) and from 40% to 75% for hepatitis B patients (25, 26). Nevertheless, in United Arab Emirates the rates were higher, 97.9% and 95.7% among patients with hepatitis B virus or hepatitis C virus, respectively (27). In addition to these findings, prevalence of TTV infection is depending on the country or area. The prevalence of TTV in Iranian patients with chronic HBV or HCV was the relatively same with the

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prevalence of this virus in United Arab Emirates and was different in Iran and other studies (23, 28). The results showed that the infection rate is significantly higher in national patients with HBV or HCV compared to that of their healthy individuals, which is in accordance with previous studies (28, 29). Also this can be attributed, in part, to the mode of transmission of the virus; the virus is transmitted not only by blood transfusion, but also by other routes such as the fecal–oral route (30). According to recent and noted studies, TTV infection is a relatively common virus infection throughout the world in different places and different racial groups and TTV is highly associated with HBV and HCV infections and therefore 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.

In recent study was investigated the impact of TTV infection on liver damage with hepatitis viral infections and healthy individuals. For this purpose, serum level of ALT was tested in all the cases. The results showed that TTV infection did not cause severe liver damage in two groups of hepatitis patients and healthy group. This was indicated by a very moderate elevation in ALT level in the disease groups, which in cirrhotic patients due to HCV showed a high ALT level than those of chronic liver disease due to HBV and HCV (31). While reviewing our findings in reference to various other studies, we found that our data are in agreement with several other reports. Previous studies stated that TTV did not modify the serology or the biochemical markers, hepatic AST and ALT levels in between individuals with hepatitis B and C viruses and healthy blood donors (23, 32). In contrast, several series of hepatitis C virus patients' coinfection with TTV appeared to be associated with increased severity of biochemical and histologic parameters of liver damage (33, 34). Also, other study indicated that children coinfected with hepatitis B virus and TTV had evidence of greater liver damage (35).

Earlier reports indicate that a majority of health individuals who become TTV-DNA-positive usually have normal ALT level and do not develop chronic hepatitis that is similar to recent study (36). We found TTV-DNA in 18% normal population with normal ALT level and at the same time no significant increase in ALT in hepatitis and B, TTV-positive patients, thereby suggesting that TTV alone does not cause much change in ALT level.

TTV infection has been found to be coinfected with HBV, HCV infections, in this study, it can be concluded that TTV is a frequent virus isolated from patients with HCV and HBV hepatitis, and from the healthy population. TTV does not significantly increase ALT level in these patients therefore has no effect on biochemical markers of associated viral hepatitis. It appears as if TTV is a benign virus acting as a bystander in the body without causing any damage of the liver.

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