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Original Article

Study on Prevalence of TTV among Cirrhotic patients due to Hepatitis B & C in Ahwaz University Hospitals during the Years 2004-2005

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Abstract

Background and Aims: Recently, a novel DNA virus was isolated from the serum of a patient with post-transfusion non A-G hepatitis and named TT virus. The aim of this study was to determine the prevalence TT virus among cirrhotic patients due to hepatitis B & C in infection Ahwaz.

Methods: The prevalence of TTV infection was studied in 41 patients with liver cirrhosis. TTV DNA was detected by semi-nested PCR. The plasma samples were tested for marker hepatitis B & C by ELISA test.

Results: TT virus was detected in 17(41.46%) of the 41 patients with cirrhotic liver disease. There were no significant difference between the subject TTV DNA in relation to sex and age. TTV positivity in cirrhotic patient infected with hepatitis B (52.9%) was higher than in similar patients infected with hepatitis C (47.1%).

Conclusion: TTV infection was highly prevalence in patient with cirrhotic hepatitis, especially in those with hepatitis B virus infection.

Keywords: Transfusion Transmitted Virus; hepatitis B virus; hepatitis C virus; Liver cirrhosis

Introduction

Cases of hepatitis that are acute and chronic have been identified caused by agent serologically and genomically distinct from the hepatitis viruses A, B, C, D, and E (1). Most patients with fulminant hepatitis are without the typical markers of hepatitis viral infection (2, 3). These findings strongly suggest the existence of additional hepatitis viruses. In 1995, GB virus C/hepatitis G virus (GBV-C/HGV) was found as a candidate for a new hepatitis virus (4, 5). Although GBV-C/HGV was first reported to be associated with fulminant hepatitis of unknown etiology, many subsequent reports have suggested no or limited pathogenicity of GBV-C/HGV as a hepatitis virus (6).

In 1997, a novel DNA virus, designated as the TT virus was cloned by representational difference analysis from an acute phase serum of a patient with post transfusion hepatitis of unknown etiology (7). The virus could be isolated from tree of five Japanese patients with post-transfusion non A to G hepatitis, and has come to be regcorded as a ″transfusion-transmitted virus″. TTV is an unenveloped negative circular single-stranded DNA virus and comprises 3.852 nucleotides, with an isopycnic density of 1.31 to 1.34 g/ml in CsCl (8, 9).

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The analysis of several open reading frames (ORFs) suggested that TTV is a member of the Circoviridae family (9). The clinical implication of TTV infection remain largely unresolved and its epidemiology is unknown (10).

The aim of this study was to determine patients due to Hepatitis B and C infection in few hospitals in Ahwaz.

Methods

Patients

From September 2004 to September 2005, plasma samples were collected from 41 cirrhotic patients (29 male, 12 female) who visited the Imam

Khomaini hospital of Ahwaz (Table1). Plasma samples were taken on admission from all patients. Plasma samples for PCR were aliquoted and stored -80ºC until analysis. follow up samples were also collected from those patients who were positive TTV DNA.

Serological tests

Type-specific diagnosis of Hepatitis viruses from cirrhotic patients was made by testing for the following hepatitis viral markers: hepatitis B surface antigens (Diaplas Inc), IgM antibodies to hepatitis B core antigen (Microwell ELISA), HBe Ag/Ab (DIA.PRO), HCV antibodies (Diaplas Inc), the samples were tested by the ELISA method.

Detection of TTV DNA

DNA was extracted from 200µl samples using a high pure viral nucleic acid kit (Roche, Germany) and was resuspended in 50µl elution buffer. TTV DNA was amplified by semi-nested PCR with TTVspecific primers derived from two conserved region of the published sequences (11). For the first

around 0.5µl primer A (sense primer NG059: 5′-ACA GAC AGA GGA GAA GGC AAC ATG-3′) 0.5µl of primer B (antisense primer NG063: 5′-CTG GCA TTT TAC CAT TTC CAA AGT T-3′) were used in 25µl PCR mixture containing: 2.5µl 10xPCR buffer, 0.5µl dNTPs, 5µl template, 0.2µl Taq DNA polymerase, 15.8µl PCR water. The amplification was for 35 cycles at 94 ºC for 30s, 60ºC for 30s and 72ºC for 45s, followed by 7min at 72ºC. In the second round with another sensepatients by hepatitis B and in group TTV-positive with cirrhotic patients by hepatitis C. primer C (NG061: 5′-GGC AAC ATG TTA TGG ATA GAC TGG-3′) and the same antisense primer B were used. The second round of PCR was performed for 25 cycles under the same time temperature conditions using 2µl of the first round PCR products as template. The PCR products (271bp) were electrophoresed in 2% agarose gel, stained with ethidium bromide, and photographed under ultraviolet light. Positive samples (blood transfusion center Tehran-Iran) and negative controls were included in each run. DNA 100bp ladder was used as DNA molecular weight size marker.

Statistical analysis

Statistical analysis was conducted using the Chi-square test. P-values of less than 0.05 were considered to be significant.

Table 1: TTV seroprevalence among individuals

Patient features	n	TTV DNA Positive (%)
Gender		
Male	29	10
Female	12	7
Age (years)		
20-29	2	1(50%)
30-39	0	0 (0%)
40-49	8	1(12.5%)
50-59	22	10 (45.45%)
60-69	9	5 (55.5%)
Total	43	17 (39.5%)

Results

Table 2: TTV DNA in sera of patients with cirrhotic liver disease

Backgrounds	Sex(M/F)	TTV DNA Positive (M/F)
Any HBV marker*	22/9	9 (4/5)
HCV antibody	7/3	8 (6/2)

* Any HBV marker includes HBs antigen, HBc antibody, HBe Ag/Ab

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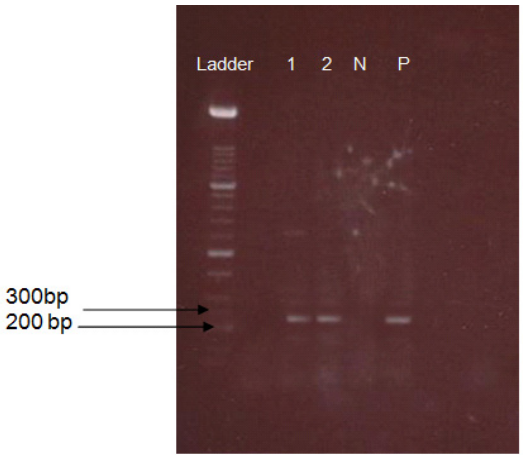

Fig. 1: Positive TTV tested samples (molecular size 271bp) confirmed by semi nested PCR method.

Presence of TTV DNA in cirrhotic hepatitis patients TTV DNA was detected in 17 of 41 patients (41.46%) with cirrhotic hepatitis (Fig1).

TTV positivity in cirrhotic patients than with hepatitis B (52.9%) was higher in cirrhotic patients (47.1%) with hepatitis C infection (Table 2).

The sex distribution was not significantly different in TTV-positive individuals with infected hepatitis B and hepatitis C viruses.

The age distribution was not significantly ifference in group TTV-positive with cirrhotic patients by hepatitis B and in group TTV-positive with cirrhotic patients by hepatitis C.

Discussion

A new DNA virus named TTV was recently identified in Japan in the serum of patients with posttransfusion non A-G hepatitis. TTV was detected in 41.46% of cirrhotic patient from Ahwaz city in Iran. The prevalence of TTV infection among cirrhotic patient in other countries has been reported to be 10% in the Korea (12), 66.7% in Japan (13), 63% in Taiwan (14). These differences in prevalence between countries could be due to the different geographical distribution of TTV infection, and the heterogeneity and variability of TTV isolates (11, 15). Variation could also arise due to different experimental methods to determine TTV infection, such as the primers used, and the sensitivity of the PCR methods employed (16, 17). The primers used in our study was identical to that used in the aforementioned countries, suggesting that the discrepancies of TTV prevalence between countries were not due to variation in the primer used.

Table 1 shows the TTV seroprevalence of subgroups of patients when these were divided by gender and age. There were no significant differences between TTV DNA in relation to sex. Vasconcelos et al. have reported similar finding (18), which were confirmed by Sioda et al (13).

With respect to age, the frequency of TTV infection was relatively high at the age of 50-59, but our results showed no difference between age distribution and TTV positive. Which are in agreement with results reported by Gad et al (19).

In conclusion TTV infection was highly prevalence in patient with cirrhotic hepatitis, especially in patients with hepatitis B.

TTV viremia is widespread with a very high incidence in general population worldwide. This suggests that TTV is a common virus and may be a nonpathogenic DNA virus in humans, although the pathogenic roles of TTV still remain to be investigated.

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