Prevalence of Torque Teno Virus in Hepatitis C Virus Infected Patients

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ABSTRACT

Background: TTV is the first human circoviridae 1997 in Japanese patients with hepatitis of unknown cause were isolated. Since then, numerous studies on the various aspects of creating the city using C virus infection have been achieved. The aim of the present study, the prevalence of TTV infection in patients with hepatitis C are two different primer pairs.

Chronic center C Materials and Methods: In this descriptive study, blood samples of 240 patients with hepatitis TTV DNA using polymerase chain reaction for the presence of clinical research professor Alborzi Shiraz and chi-square test using SPSS two different primer pairs were studied. Results were analyzed using the software.

5 and 12 samples (5-UTR Results: Of the 240 patients, 220 patients (92%) were positive by the primers. Based on demographic data, the difference between the amount of virus N %) of the 22 TTV primers by there were male and female.

Chronic 5-UTR region using primers C prevalence of TTV infection in patients with hepatitis prevalence of high N was almost in agreement with studies in other countries, but using primers to the lower 22 come. Overall, there was no significant association between sex and the prevalence of the virus. Prevalence controversial or need more studies on the association between the viruses among patients with hepatitis C virus is even higher.

KEYWORDS: Iran, TTV, hepatitis virus C.

INTRODUCTION

(TORQUE TENO-virus family and gender Syrkvvyrydh TTV), was the first human virus Syrkv 1997 by the late Mr. Nishizawa in Japan Nyshy 3 patients with serum HBV DNA was isolated with the unknown (Nishizawa et al., 1997). This organism is a single-stranded non-enveloped viruses. Ayjadhaty and aplastic anemia is thought that the virus is the virus responsible for hepatitis from a blood transfusion is sometimes known. The PCR test to detect the virus from samples definitive diagnostic confirmation is used. Infection with this virus have been reported in many countries (Hu et al., 2005). Genotype distribution in the geographic areas of the world are still not completely understood (Dai et al., 2000). Design primers for 5'-UTR are most genotypes are identified, the specific identification of N 22 can have a limited number of genotypes (Zehender et al., 2001). Various studies have reported different prevalence in a given society is probably one of the reasons why it is different primers. One outstanding feature of TTV genetic diversity, so much silt that it is the same today, more than 30 genotypes have been classified.

The N22 ORF 1 genotype analysis in many viruses have been identified that differ by more than 30% (Okamoto et al., 1999). Transmission of blood-borne virus, transmitted through the placenta and is digestive, respiratory and sexually transmitted addition it has been suggested (Werno et al., 2000). Virus DNA in saliva, tears, semen, throat swabs, cerebrospinal fluid - CSF and in several human tissues, including liver, bone marrow, peripheral blood mononuclear cells of infected individuals is (Pourshams et al., 2004). TTV is able to temporarily or chronically infect their hosts, but its role as a human pathogen is still controversial. Seems that the high genetic diversity of the virus, allowing the pathogen of the virus alone a certain amount of genotypes or (Krekulova et al., 2001). The purpose of the study, the prevalence of TTV infection in patients with chronic hepatitis C using 2 different primer.

MATERIALS AND METHODS

This research is a descriptive study of the TTV First as a virus. Effective potential in the pathogenesis of hepatitis was introduced during the years 1378-1388 on samples of patients with chronic hepatitis c Professor of Clinical Microbiology Research Center, Shiraz Alborzi already proven susceptible to chronic hepatitis C were
(Table 1). All samples were previously positive for hepatitis C infection was confirmed by the reaction. After blood collection, plasma samples immediately in the laboratory of Professor of Clinical Microbiology Research Center, Shiraz Alborzi isolation and were stored at minus 70 degrees Celsius. Samples of Shiraz in compliance with the cold chain, the freezer minus 70 °C were transferred to the Department of Virology University. DNA viruses than 200 micro liters of plasma was extracted using a kit from DNA (DNA Mini Kit Q Vyrvsy the AH load requirement, Inc. America Qiagen payments other Issue: 52904). Was extracted according to the manufacturer’s instructions. This study was approved by the Medical Ethics Committee for Biosafety and the University. All patients provided informed consent form to participate in the study and collected.

<table>
<thead>
<tr>
<th>Age Range of sample</th>
<th>woman</th>
<th>man</th>
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<tbody>
<tr>
<td>14-24</td>
<td>7</td>
<td>31</td>
</tr>
<tr>
<td>24-34</td>
<td>11</td>
<td>72</td>
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<td>34-44</td>
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<td>37</td>
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<tr>
<td>44-54</td>
<td>3</td>
<td>49</td>
</tr>
<tr>
<td>54-64</td>
<td>2</td>
<td>20</td>
</tr>
<tr>
<td>total</td>
<td>31</td>
<td>209</td>
</tr>
</tbody>
</table>

**The age range of subjects**

Primers selected for this study are shown in Table 2. These primers UTR and ORF1 genes of the virus are connected. Total length of about 300 nucleotides in length gene UTR genotype 1ORF, 2300 base pairs. During the final product of gene amplification UTR and ORF of 220 and 270 base pairs, respectively. In this study, the size marker (Marker Ladder, 100bp Fermentase company) was 100 bp. Specific primers based on the alignment of 200 sequences of UTR and ORF1 gene bank database (GenBank) Mega 4 software was selected. In addition to the reference sequences in the gene bank (002076.2_NC) was used as the reference sequence.

**Table 2. Nucleotide sequences of primers used in this study to detect TTV**

<table>
<thead>
<tr>
<th>sequences of primers</th>
<th>Location identified</th>
<th>Primers name</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-ACA GAC AGA GGA GAA GCC AAC ATG-3</td>
<td>N22</td>
<td>NG054</td>
</tr>
<tr>
<td>5-GGC AAC ATG YTR TGG ATA GAC TGG-3</td>
<td>N22</td>
<td>NG061</td>
</tr>
<tr>
<td>5-CTG GCA TTT TAC CAT TTC CAA AGT T-3</td>
<td>N22</td>
<td>NG063</td>
</tr>
<tr>
<td>5-TTT GCT ACG TCA CTA ACC AC-3</td>
<td>5-UTR</td>
<td>NG054</td>
</tr>
<tr>
<td>5-GCC AGT CCC GAG CCC GAA TTG CC-3</td>
<td>5-UTR</td>
<td>NG147</td>
</tr>
<tr>
<td>5-AGC CCG AAT TGC CCC TTG AC-3</td>
<td>5-UTR</td>
<td>NG132</td>
</tr>
</tbody>
</table>

**Primer sequences recognized place name primer**

The method used for the amplification of viral DNA the 5-UTR in Semi-nested-PCR, which uses a new combination of primers NG054 and 132NG, NG147 was the case for the first-round reaction Azpraymhray NG054 and NG 132 was used, since the combination of different primers and primers used in this study of virus -specific primers were more capable of TTV sequences were selected and put together . UTR of the gene product size 300 bp and 220 bp was amplified in the second round (Fig. 1).

**Figure 1. TTV positive bp 220 Band GENE and negative control samples**

In the optimization stage of the reaction, distilled water, negative human genome and viral hepatitis B, G, K and AIDS as a negative control and positive control samples of four TTV infection confirmed by positive blood donors were used. Primers on samples of this study was to assess the likely positive and negative controls as well as non-
specific binding to either the genome of hepatitis B, C, G and human genomes were evaluated. Other components, such as PCR and primers concentration, reaction temperature, number of cycles of binding reactions by several gradients of these components were optimized (Figure 2).

After optimization of the reaction, the performance of PCR in the first round, 5 microliters of DNA template (approximately 150 ng), 0.75 ml of each of the primers external (solution of 10 mM), 5 mold NTP mix (10 ml) with a final concentration of 2 Micro M, taqDNA final concentration of 4 IU polymerase (Fermentas, Build Number: 0402 EP). One microliter of MgCl2 10 mM, Fermentas, Build Number: R0971), the semi-micro-molar concentrations, and two and a half microliters of PCR 10X buffer with a final concentration of 1X, (Ntaz form companies, build number: B38) was determined. The method used for viral DNA replication, as well as the N22, Semi-nested PCR was used to Azpraymrhay NG063, NG061 and NG59 were conducted in two phases and the first phase of the primers NG061 NG59, NG59 and NG063 were used in the second stage.

Product size 286 and 270 bp, respectively, first and second respectively. Material volume and reaction buffers at the same stage of the reaction for 5-UTR gene mentioned above was given to the program, Thermal devices cycler was as follows: 7 min at 95 ° C for one cycle, 30 seconds at 94 ° C, 45 seconds at 57 ° C, 50 seconds at 72 ° C for 30 cycles and finally 22 ° C for 7 min. The second stage is the first stage of the program is similar except that the number of cycles was reduced to 25 cycles at a rate of 2 microliters of the reaction pattern of the product of the first stage. There and see the bands 200 and 270 bp, indicating the presence of TTV. This study is a descriptive study and experimental sampling random is, a total of 240 infected with hepatitis C along with demographic data, five negative control samples of human and 5 samples of each virus, hepatitis G, and B and HIV study were. Data for the survey design and statistical analysis, SPSS software and chi-square tests were used.

RESULTS

With demographic information and test results are routinely reviewed, 240 patients participated in the study, 209 were male and 31 were female. The overall average age of 35/83 years, the men and women of this parameter for the 36/1 and 33/21 years of infection with hepatitis C minimum and maximum ages of 4 and 64 years, respectively. Hepatitis C infection, respectively, 4 and 64 years. Most patients with TTV infection in age groups 24-34 and 54-65 years had the lowest. With the normalization of demographic data would seem that the rate of hepatitis C virus infection in men and women is almost equal (Figure 1).
After optimization, the method is designed on 240 cases of hepatitis C positive, 10 negative control samples of human genome and 5 samples of each virus, TTV, hepatitis G, C, and B to determine the attributes of the analytical, the installation procedure was performed. Working as a negative control in each round of sterile distilled water, along with the samples, was used. The experiments were performed using primers 5-UTR and N22, respectively, 192 and 12 out of 240 bands were detected in the sample. None of the negative control samples of human viral and no band was observed. The positive samples were non-specific bands.

DISCUSSION

TTV is a new virus that Nyshy Nishizawa in 1997 by a Japanese patient with hepatitis of unknown etiology, were isolated. The first time, with increased levels of alkaline phosphatase activity were reported. It was thought that TTV is a virus and hepatitis (Okamoto et al, 1999). Potential transmission through blood and blood products and a high level of virus in the liver, as well as isolation of patients with unknown hepatitis virus, causing the need for the population prevalence of the virus in blood donors and patients with hepatitis is (Zandi et al, 2006).

Phylogenetic studies showed that many of these viruses are genotype and co-infection with multiple TTV infection, especially in people with thalassemia have been reported frequently. Perhaps one of the reasons for high levels of TTV infection in patients with thalassemia major, de Lille has received numerous blood and blood products (Werno et al, 2000).

TTV genetic variation is a big problem for the detection of virus by PCR is 2. Avkamvtv reported that the primers ORF-1 was first detected TTV isolate (TA278) cannot identify the American isolate TUSO1 with other primer was identifying isolates (Okamoto et al, 2001). The virus genome was identified as a high genetic variation in genomic DNA viruses, however, the amount of genetic variation has been observed (Werno et al, 2000).

Avkamvtv reported primers ORF1 region was first detected TTV isolate (TA278) can not identify TUSO1 American isolates with primers to detect if these isolates were identified (Jalali Far et al, 2007).

The virus genome was identified as a high genetic variation among genomic DNA viruses, however, the amount of genetic variation has been observed (Desai et al, 1999) . So to know the actual prevalence of the virus
from the system, it must be designed primers for a conserved region of the virus used. Although the virus genome is very heterogeneous, but the UTR-5 below the TATA box located between genotype somewhat protected, so the primers used in this study for the 5-UTR were selected (Moreno et al, 2004). TTV 42.5, 28, 37, 62 and 19 percent, respectively, in blood donors in Italy, Turkey, China, Thailand, Iceland and Scotland there (Prescott et al, 1999).

The virus prevalence among the general population in Japan, 12-35% have been reported (Baba et al, 2006). A study in Japan, the prevalence of TTV infection among blood donors (12%) reported an incidence of about 9 percent in the next Takashi population of healthy individuals between the two studies reported in Japan, mainly in system of primers used for (Krekelova et al, 2001). Using the primers N22, NG061 and NG063 G different incidence rates in different regions of Iran as the following have been reported in blood donors in Tehran 66/9% of Tabriz in hemodialysis patients and blood donors, respectively, and 2/7 and 9/3 percent, respectively, in patients with thalassemia major Ahwaz blood donors 57/2 and 23/7% and has been reported (Yuki et al, 1999). In America and Japan also with primers corresponding outbreaks (10%) has been calculated (Dai et al, 2000) and in a study in Tehran prevalence of TTV infection in patients with chronic hepatitis C, 18/9 percent were amplified by the 5-UTR has been reported (Nishizawa et al, 1997).

Overall, it appears that the prevalence of infection in blood donors in Iran of Blood Donors in America (1%) and Western European countries are infected with the virus, similar contamination of blood donors in China (28 percent) but lower than Thailand (37%) and Italy (42%) (Yuki et al, 1999). More studies in this review are whether TTV infection is associated with liver disease or not? Hepatitis C with the previous studies that also were TTV TTV co-infection with hepatitis C and 30 to 88% were reported to be confirmed (Moreno et al, 2004).

In the study area virus outbreaks with primers N22 (5%) and a lower incidence of virus amplified by the 5-UTR (92 percent) in the other studies, including a study of the prevalence of the virus among patients in America chronic hepatitis C region primers N22 and 5-UTR5 respectively 29 and 70% reported almost are in agreement (Desai et al, 1999). So select the part of the gene and the prevalence of TTV has a significant effect on the prevalence rate in this study was obtained using the primers N22 from 5% to 92% using the primers 5-UTR in a similar study in Saudi Arabia reached the level of 19% to 100% (Bendinelli et al, 2001). But different tables spread across the different primers used, may be related to differences in population demographics such as age or the presence of different genotypes in different regions is. Areas are different, and therefore the incidence of virus the N22 is not right but since the primers district 5-UTR are capable of all genotypes identified so the incidence of more virus must primers for the 5-UTR is used.

In this study, the prevalence of the virus was found between age and gender, which is consistent with some studies (Jalali Far et al, 2007). Although other studies related to age or sex prevalence has been reported (Baba et al, 2006).

Conclusions

According to the results of the present study indicate that the prevalence of TTV infection in patients with hepatitis C in Iran is higher than other studies done. Due to the nature of the virus of unknown cause more harm to pursue the consequences of infection with this virus is necessary.

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REFERENCES


