TTV infection in children with and without liver disease
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Background: TTV DNA has been reported in patients with a broad spectrum of hepatic disorders as well as in healthy people. Aim: To clarify the role of TTV in children with liver disease and in healthy children. Methods: Degenerate primers designed to amplify a target sequence from the ORF 1 region of TTV genome were used for nested PCR, to detect TTV DNA in sera. Results: TTV was detected in 3 of 18 children with chronic hepatitis B (16.7%), 2 of 17 hepatitis B carriers (11.8%), 2 of 17 children with cryptogenic chronic liver disease (11.8%), and 1 of 40 (2.5%) children without liver disease. The infection rate was similar among the various study groups and in the various age groups. There was no difference between TTV positive and negative children in respect to gender, history of surgery, parenteral treatment, transfusion of blood and blood products, presence of hepatomegaly, splenomegaly, jaundice, and transaminase values. Conclusion: TTV does not seem to have an etiologic role in cryptogenic liver disease in children and does not seem to influence the clinical course of liver disease. [Indian J Gastroenterol 2004;23:135-137]

Key words: Chronic hepatitis B, cryptogenic liver disease, hepatitis B

In 1997 a single-stranded non-developed new DNA virus was identified in patients who were diagnosed to have post-transfusion hepatitis, and was named as the TT virus.1 Several studies showed the presence of TTV DNA in patients with a broad spectrum of liver disorders, such as acute hepatitis, chronic hepatitis B and C, fulminant hepatic failure, cryptogenic cirrhosis, as well as in healthy persons and blood donors.2-9 However, the association between TTV and hepatitis remains doubtful. We evaluated the frequency and significance of TTV in children with cryptogenic liver disease and chronic hepatitis B infection.

Methods
Ninety-two children (aged 1.5-17 years, mean [SD] 9.8 [4.2] y; 54 male) admitted to University Pediatric Gastroenterology Unit between May 2000 and September 2000 were studied. They included 52 with liver disease (18 chronic hepatitis B, 17 asymptomatic hepatitis B carriers, and 17 cryptogenic liver disease) and 40 children with no evidence of liver disease clinically or biochemically. None of these patients had history of antiviral or immunomodulatory therapy. Informed consent was obtained from the parents or guardians of the children. Children with normal ALT and AST levels, and with hepatitis B surface antigen (HBsAg) and hepatitis B e antibody (anti HBe) positive, and negative serology for hepatitis B virus DNA (HBV DNA) and anti-delta were defined as hepatitis B carriers. Children with normal-high ALT and AST levels and with HBsAg, HBeAg and HBV DNA positive, negative serology for anti HBe and anti delta were defined to have chronic hepatitis B. Those with negative serologic tests for hepatitis A-E, cytomegalovirus, Epstein-Barr virus, toxoplasmosis, rubella, herpes virus 1 and 2 as well as autoantibodies, and with abnormal liver biochemistry or other evidence of chronic liver disease were labeled as having cryptogenic liver disease. Wilson’s disease, cystic fibrosis, α-1 antitrypsin deficiency, and metabolic liver diseases were differentiated by appropriate tests when clinically suspected.

In the control group, forty children who had normal serum transaminase levels with no clinical evidence of liver disease and who were negative for HBsAg and anti HCV were included. These children were being evaluated for gastroesophageal reflux, exogenous obesity, functional abdominal pain, and chronic constipation. History of blood or blood product transfusion, surgery and parenteral treatment were asked for at the time of venous sampling.

Assays for HBsAg, anti HBs, HBeAg, anti HBe, anti HCV and anti delta were done using commercial radioimmunoassays (Axyme, Abbott Laboratories). HBV DNA was determined quantitatively by a molecular hybridization assay (Digene Diagnostics; normal level <5 pg/ml).

Detection of TTV DNA
All sera collected for the study were stored at -80°C prior to testing. DNA extracted from 200 microliters of serum using the Viogene DNA-RNA extraction kit (Sunnyvale, CA, USA) was resuspended in 50 μL of distilled water: 10 μL of DNA was subjected to PCR in a 50 μL reaction mixture containing PCR buffer (2 mM MgCl2), 0.5 mM dNTPs, 0.5 μM Taq polymerase (Promega, USA). Degenerate primers designed to amplify a target sequence from ORF 1 region of TTV genome were used for the nested PCR protocol.

For the first round, sense primer TT1 (5'-CAG ACA GAG GAG ACA ACA TC-3') and antisense primer TT2 (5'-TAC CAY TTA GCT CTC TAT TCT WA-3') were

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Table 1: Clinical and laboratory characteristics of TTV DNA-positive and -negative groups

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<thead>
<tr>
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<th>TTV DNA Positive (n=6)</th>
<th>TTV DNA Negative (n=54)</th>
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<tbody>
<tr>
<td>Age (y)</td>
<td>9.3 (4.3)</td>
<td>9.9 (4.3)</td>
</tr>
<tr>
<td>Gender (female: male)</td>
<td>5:3</td>
<td>33:51</td>
</tr>
<tr>
<td>Transfusion history</td>
<td>2</td>
<td>2 (9.5%)</td>
</tr>
<tr>
<td>Parenteral treatment</td>
<td>2</td>
<td>12 (14.3%)</td>
</tr>
<tr>
<td>Surgical intervention history</td>
<td>1</td>
<td>5 (9.5%)</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>23.1 (9.5)</td>
<td>55.7 (74.9)</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>51.2 (10.4)</td>
<td>68.9 (74.9)</td>
</tr>
<tr>
<td>HBsAg positive</td>
<td>5</td>
<td>31 (36.9%)</td>
</tr>
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Values as mean (SD); p<0.05 for all
d

used for the amplification of the target sequence of 326 base pairs (bp). For the second round, sense primer TT3 (5' GGCAATGACCTGACGGGTAATGGTGG-3') and antisense primer TT4 (5' GACCGGTTTACGCATGAG-3') were used for a 227-bp target sequence. MgCl2 concentration was increased to 2.5 mM for the second round. Each reaction was carried out for 35 cycles at 94° C for 30 seconds, at 60° C for 45 seconds, and finally 10 min extension at 72° C in a thermal cycler. Amplicons were electrophoresed in 2% agarose gel and observed with ultraviolet light after staining with ethidium bromide. For each round of PCR, negative and positive control sera were included in the reaction.

Statistical analyses

Groups were compared using χ² and Mann Whitney U tests at an α level 0.05.

Results

The patient (mean [SD] age 10.3 [4.2]; y: 31 male) and control (9.2 [4.2]; y: 23 male) groups were similar in age and gender distribution. TTV DNA was detected in 7 of 52 (13.5%) patients—3 chronic hepatitis B, 2 hepatitis C carriers, 2 cryptogenic liver disease—and only 1 of 40 controls (2.5%) (p<0.05). The frequency of TTV positivity was similar in children infected with HBV or those without (5/30 vs 2/56; p<0.05). There was no difference between TTV DNA-positive and -negative patients with respect to age, gender, history of surgery, parenteral treatment, transfusion of blood and blood products, clinical features, and levels of serum ALT and AST (Table 1). There was also no difference in liver function test levels between isolated HBsAg-positive patients and patients who were both TTV DNA and HBsAg positive (Table 2).

Discussion

The prevalence of TTV was found to vary widely in the healthy population in different countries. An epidemiological survey performed in our hospital utilizing the same set of primers for ORF 1 detected TTV in 40% of the healthy adult population (unpublished data). The impact of TTV on liver damage in liver diseases is controversial. Although some studies point to a significant increase in the prevalence of TTV in chronic cryptogenic liver disease, no difference between the diseased and healthy control groups has been reported in other studies.

The present study attempts to clarify this relationship in children. In our study, TTV DNA was detected in 16.7% of chronic hepatitis B patients, 11.8% of asymptomatic hepatitis B carriers, 11.8% of patients with cryptogenic hepatitis, and 2.5% of the control group. Despite the small numbers in each group, our study gives an idea about the frequency and probable role of TT virus in chronic liver disease in children in our country. The lower prevalence of TTV DNA in our study may be due to the age group studied, regional differences, or the sensitivity of the primers used during PCR.

One study in Turkey detected TTV infection in 34% and 6% with two different primer sets in the same 50 chronic HBV patients, while the infection rate in 150 healthy blood donors was 40% and 8%, respectively.

Sakac et al. reported an increase in prevalence of TTV with age; they did not provide information about the prevalence in children under 11 years. We found no difference in prevalence of TTV from 1.5-17 years of age. This is similar to the finding reported by Iriyama et al. from Japan. Transplacental transmission of TTV has been suggested, based on similarity of viral sequences in mother-infant pairs.

We found no difference in ALT and AST levels between the TTV DNA-positive and -negative groups. Sixteen genotypes of TTV DNA have been identified; it is possible that only some of these are pathogenic to the liver. Okambara et al. found 30% positivity of TTV DNA in patients with acute and chronic liver diseases. They showed a high prevalence of genotype 1a in this group. It has been shown that genotype 1a and 1b have different characteristic nucleotide and amino acid sequences.

Controversy exists about the association of chronic hepatitis B infection and TTV. We found no difference in TTV DNA prevalence between HBsAg-positive and -negative cases, in contrast to Germer's study. Liver transaminase and alkaline phosphatase levels were

Table 2: Liver function tests in isolated HBsAg positive and HBsAg and TTV DNA positive patients

<table>
<thead>
<tr>
<th></th>
<th>HBsAg (+) (n=5)</th>
<th>HBsAg (-) (n=54)</th>
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<tbody>
<tr>
<td>ALT (IU/L)</td>
<td>22.0 (8.4)</td>
<td>46.9 (40.2)</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>31.6 (9.7)</td>
<td>41.2 (26.1)</td>
</tr>
<tr>
<td>ALP (IU/L)</td>
<td>550.8 (151.1)</td>
<td>589.9 (285.7)</td>
</tr>
<tr>
<td>GGT (IU/L)</td>
<td>13.2 (2.7)</td>
<td>23.0 (3.9)</td>
</tr>
<tr>
<td>p-value</td>
<td>p&lt;0.05</td>
<td>p&lt;0.05</td>
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not different in HBsAg-positive subjects as compared with subjects who were both HBsAg and TTV DNA positive. This finding suggests that TTV does not affect the severity and course of chronic hepatitis B infection.

Long-term follow up studies are needed to determine the prevalence of TTV in different age groups, its possible role in chronic liver diseases, the most common genotype, and the effect on the clinical course of liver diseases.

References


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