Presence of TT virus infection in chronic hepatitis patients from a hospital in New Delhi, India

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Background & objectives: The recent discovery of a novel parenterally transmitted, unenveloped, single-stranded DNA virus called TT virus (TTV) in chronic hepatitis patients with unclear pathogenesis throughout the world led us to investigate, its presence in chronic hepatitis patients attending a hospital in New Delhi, India, and to evaluate its role in liver disease.

Methods: TT virus DNA was investigated in serum samples of 70 patients with various types of chronic hepatitis, and 100 healthy subjects from New Delhi, India by nested PCR using the primers that belonged to UTR (A) region of the genome.

Results: TTV DNA was detected in 6 of 23 patients (26%) with type B chronic hepatitis, 3 of 20 patients (15%) with type C chronic hepatitis, and 12 of 100 subjects (12%) from healthy control group with normal liver function profile tests. None of the 27 non-B, non-C chronic hepatitis patients had TTV DNA positivity. The prevalence of TTV was significantly higher in type-B chronic hepatitis patients as compared to normal subjects (P < 0.05) but comparable to type C chronic hepatitis patients. The clinical course and biochemical profiles of type B, or type C chronic hepatitis patients co-infected with TTV did not differ significantly from those without TTV infection.

Interpretation & conclusions: Interestingly, in chronic hepatitis patients, TTV was always associated with either hepatitis B or C virus indicating a likely parenteral route of transmission. All TTV-positive subjects in healthy control group showed normal clinical and biochemical profiles. Thus, the presence of TTV infection is unlikely to influence the course of chronic hepatitis related to hepatitis B virus (HBV) or hepatitis C virus (HCV) or cause liver diseases in healthy subjects.

Key words Polymerase chain reaction - TT virus - type-B hepatitis - type-C hepatitis

Throughout the world there still remain patients with chronic liver diseases unexplained by hepatitis C or B viruses (HCV or HBV)1-6. The discovery of hepatitis G (HG) virus failed to show that this virus was the major cause of non-B, non-C chronic hepatitis7. Recent discovery of TT virus (TTV), a non enveloped single-stranded circular DNA virus from Japan has rekindled the hope of a hepatotrophic virus that may be able to explain the non-B, non-C chronic hepatitis cases2,5. TTV is a negative-stranded circular DNA virus measuring 30-50 nm that belongs to the Circoviridae8. Prevalence of TTV in chronic hepatitis patients and voluntary blood donors in western India was found to be 6.7 and 7.4 per cent respectively1,
but TTV was not found to be an important cause of chronic liver diseases. Information regarding the prevalence and clinical significance of TTV in viral hepatitis patients from northern India is scanty. We investigated the presence of TTV DNA in serum samples of patients with chronic hepatitis attending a hospital in New Delhi, India and healthy subjects, and the possible role of TTV in non-B, non-C chronic hepatitis was also evaluated.

Material & Methods

Patients: A total of 70 consecutive patients with biopsy proven chronic hepatitis, who visited the Out Patient Clinic of Lok Nayak Jai Prakash Hospital, New Delhi between July 2001 and June 2002, were included in the study. Serum samples were taken as baseline followed by samples taken at 1-3 months intervals. Patients were diagnosed as having chronic hepatitis, using conventional clinical, biochemical and histological criteria. The mean duration of the disease was 13 ± 4 months. Anti-HCV or HBsAg positive patients with persistently elevated alanine amino transferase (ALT) levels (>60 IU/l), detectable HCV RNA or HBV DNA in serum, and a liver biopsy that indicates either portal or bridging fibrosis or at least moderate degree of inflammation and necrosis were included under HCV or HBV related chronic hepatitis categories. Patients with autoimmune liver disease, drug-induced hepatitis and alcoholic liver injury were excluded. A group of 27 patients (chronic non-B, non-C hepatitis) was seronegative for both HBV and HCV markers as revealed by ELISA and PCR studies. History of blood transfusion (HBT) was recorded in 8 patients in this group. Another group of 23 patients (chronic hepatitis B) was seropositive for HBsAg and HBV DNA and seronegative for anti-HCV and HCV RNA. Six patients had HBT in this group. A total of 20 patients (chronic hepatitis C) were seropositive for anti-HCV and HCV RNA and seronegative for HBsAg and HBV DNA. Eleven patients in this group had HBT. 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. Twenty individuals had HBT in this group. The TTV positivity in controls was found to be 52 per cent in another study. Allowing a difference of 20% ± 10 it was found that at 95 per cent confidence limit 96 samples would be needed for the study. We therefore, included 100 controls in this study.

Serology: Serum samples collected from all 70 chronic hepatitis patients and 100 healthy control subjects were included for serological tests for hepatitis B and C viruses viz., HBsAg using Elisaan micro ELISA strips (Ranbaxy Diagnostics, England), Anti-HCV by Innogenetics NV, Ghent, Belgium.

Detection of TTV DNA: Total DNA from 100 µl serum was extracted by standard proteinase-K/phenol/chloroform method and was subjected to PCR. TTV DNA was detected in serum by nested PCR using the primers described by Tanaka et al that belonged to UTR (A) region of the genome. Briefly, DNA was amplified by PCR with 0.75 Units of Taq DNA polymerase (Bangalore Genei, India) for 35 cycles at 94°C for 30 sec, 55°C for 30 sec and 72°C for 45 sec, followed by 7 min extension at 72°C after the last cycle, in a 100-PTC thermal cycler (MJ Research, USA).

The first and second round primers were as follows:

First round primers:

NG-133 sense: 5'-GTAAGTGCACCTCCGAATGGCTGAG-3'
NG-147 Anti-sense: 5'-GCCAGTCCCGAGCCCGAATTGCC-3'

Second round primers:

NG-134 sense: 5'-AGTTTTCCACGCCCCTGCACTGAG-3'
NG-132 Anti-sense: 5'-AGCCCCGAGTGACGGCGGCTTT-3'

The final PCR product of 110 base pairs was visualized with UV-Transilluminator (Vilber Lourmat, France) after electrophoresis on a 2 per cent agarose gel stained with ethidium bromide.

Statistical test: The statistical tests included analysis of variance (ANOVA), Bon-feronni test of multiple comparisons, Chi-square/Fisher exact test and student t-test wherever applicable. P values less than 0.05 were taken as statistically significant.
Results & Discussion

Presence of TTV DNA in chronic hepatitis patients and healthy controls: TTV DNA was detected in 9 of 70 patients (12.8%) with chronic hepatitis, which was not significantly different from the controls (12 of 100, 12%). TTV positivity in patients with hepatitis B (26%) was significantly higher ($P < 0.05$) than in controls (12%), but was comparable to hepatitis C patients (15%) (Table I). Mean age of patients with chronic hepatitis B was not significantly different than chronic hepatitis C or non-B, non-C hepatitis. We could not associate blood transfusion history as a risk factor for transmission of TTV in hepatitis B ($x^2$, $P=0.73$ CI 0.22-4.50 95% CI) and non-B, non-C hepatitis although chronic hepatitis C patients had a significant association with history of blood transfusions ($x^2$, $P=0.009$) (Table I). Mean histological activity index (HAI) scores were not significantly different among the various groups of patients. Mean ALT levels were not significantly different among various groups of patients (Table II).

Clinical differences between the TTV-positive and TTV-negative groups with chronic hepatitis: The age distribution between TTV-positive and TTV-negative patients did not differ significantly (Table II). The proportion of patients with blood transfusion histories was higher in the TTV-positive group than in TTV-negative group. The sex distribution was not significantly different between TTV-positive and TTV-negative groups. Also, mean ALT levels between TTV-positive and TTV-negative groups did not differ significantly (Table II).

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

<table>
<thead>
<tr>
<th>Study groups</th>
<th>n</th>
<th>Age (yr) (mean±SD)</th>
<th>Sex M:F</th>
<th>Mean HAI score</th>
<th>Blood transfusion history (BTH)</th>
<th>Positivity TTV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type non-B, non-C</td>
<td>27</td>
<td>44.59±19.1</td>
<td>13:14</td>
<td>13.5±4.11</td>
<td>8 (29.6)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Type B</td>
<td>23</td>
<td>43.4±10.3</td>
<td>11:12</td>
<td>12.62±2.77</td>
<td>6 (26.0)</td>
<td>6 (26)</td>
</tr>
<tr>
<td>Type C</td>
<td>20</td>
<td>46.61±14.7</td>
<td>11:9</td>
<td>14.22±6.91</td>
<td>11 (55)</td>
<td>3 (15)</td>
</tr>
<tr>
<td>Healthy control</td>
<td>100</td>
<td>42.5±8.08</td>
<td>54:46</td>
<td>Not done</td>
<td>20 (20)</td>
<td>12 (12)</td>
</tr>
</tbody>
</table>

$x^2$ - BTH in type C and TTV positivity $P=0.009$

### Table II. Clinical details of the TTV-positive and TTV-negative cases

<table>
<thead>
<tr>
<th>Study groups</th>
<th>Age (yr)</th>
<th>Sex M:F</th>
<th>History of blood transfusion</th>
<th>ALT (IU/l) (mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatitis non-B, non-C</td>
<td>N = 27</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>TTV-positive</td>
<td>44.6 ± 19.1</td>
<td>13:14</td>
<td>8/27</td>
<td>41.6 ± 19.17</td>
</tr>
<tr>
<td>TTV-negative</td>
<td>44.7 ± 9.48</td>
<td>4:2</td>
<td>4/6</td>
<td>95.15 ± 11.88</td>
</tr>
<tr>
<td>Hepatitis B</td>
<td>N = 23</td>
<td>-</td>
<td>6/23</td>
<td>-</td>
</tr>
<tr>
<td>TTV-positive</td>
<td>48.4 ± 13.5</td>
<td>1:2</td>
<td>3/3</td>
<td>106.44 ± 26.9</td>
</tr>
<tr>
<td>TTV-negative</td>
<td>40.82 ± 15.98</td>
<td>10:7</td>
<td>8/17</td>
<td>102.19 ± 14.82</td>
</tr>
<tr>
<td>Healthy control</td>
<td>N = 100</td>
<td>-</td>
<td>20/100</td>
<td>-</td>
</tr>
<tr>
<td>TTV-positive</td>
<td>41.1± 4.4</td>
<td>8:4</td>
<td>12/12</td>
<td>14.0 ± 6.6</td>
</tr>
<tr>
<td>TTV-negative</td>
<td>46 ± 11.77</td>
<td>46:42</td>
<td>8/88</td>
<td>19.17 ± 2.18</td>
</tr>
</tbody>
</table>
In the present study, the prevalence of TTV in patients with chronic liver diseases (12.8%) was found to be similar to that in healthy blood donors (12%). Arankalle et al\textsuperscript{1} reported TTV positivity only in HBV DNA positive subset of chronic hepatitis patients. However, detailed clinical data of the patients like age, sex, history of blood transfusions were not studied. Several studies have revealed high (1.9 to 36%) TTV DNA prevalence in healthy control groups\textsuperscript{6,12-18}. Interestingly, in the present study TTV when present in chronic hepatitis patients was always associated with either HBV or HCV. Also, the presence of TTV infection in our study population had no significant impact on the clinical implications of patients with chronic hepatitis. Thus, the clinical significance of TTV could not be ascertained in chronic hepatitis cases.

In conclusion, our study did not indicate TTV as a causative agent of non-B, non-C chronic hepatitis cases studied at our centre and TTV might be an innocent bystander, which is unlikely to influence the course of the disease.

Acknowledgment

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References


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