TT VIRUS INFECTION IN ACUTE NON-A TO E HEPATITIS IN NORTHERN THAILAND

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Abstract. TT virus is a novel DNA virus widely distributed in the general population. We examined the prevalence of TTV infection in a population with acute non-A to E hepatitis and in comparison groups located in Northern Thailand. The prevalence of TTV in subjects with non-A-E hepatitis was 19% (21/112), 6% (4/72) in healthy volunteers, 17% (12/72) in those with hepatitis A or B, and 17% (8/48) in hospitalized patients with non-hepatitis illnesses. A significant association with TTV infection and non-A-E hepatitis was seen in all groups (OR 3.9, p = 0.02) and in children (OR 25.8, p = 0.001). Among subjects with non-A-E hepatitis, TTV was associated with higher alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels (significant for AST, p = 0.02). Our observations suggest that TTV in our study population may be associated with non-A-E hepatitis and that children in particular may be at risk of hepatocellular injury as a result of TTV infection.

INTRODUCTION

TT virus (TTV) is a novel DNA virus that was first isolated from a Japanese patient (TT) with post-transfusion non-A to G hepatitis (Nishizawa et al, 1997). TTV is a non-enveloped, circular single-stranded DNA virus of 3,852 nucleotide length (Okamoto et al, 1999; Springfield et al, 2000). The genomic structure of TTV is divided into a noncoding region and a coding region consisting of two major open reading frames (ORF) (Okamoto et al, 1999). TTV has marked genomic heterogeneity, with at least 11 genotypes identified (Okamoto et al, 1999). Genotypes 1, 2 and 3 are distributed worldwide; genotype 1 is the most common genotype in Asia (Gallian et al, 2000). Recently, five new groups of TTV sequences have been identified as closely related but different viruses (Khudyakov et al, 2000). Current evidence suggests that TTV is a member of the family Circoviridae or a recently-established virus family, Circinoviridae (Springfeld et al, 2000; Takahashi et al, 1998).

Previous studies have demonstrated that TTV is widely distributed in the general population: approximately 2% of blood donors in the United Kingdom, 12% of donors in Japan and 6.2% of healthy Icelandic donors have been shown to be viremic for TTV (Prescott and Simmonds, 1998; Love et al, 2000). High prevalence rates of TTV in tropical countries have been cited: 83% in Gambia; 58% of post-partum women in the Democratic Republic of Congo and 54% in their infants (Davidson et al, 1999). In Taiwan, the prevalence of TTV in prostitutes was 32.9% compared with 21.3% in the control group (Huang et al, 2000). In Bangkok, Thailand, TTV was detected in 18%
of chronic non-A-G hepatitis patients with liver disease, in 9.2% of hepatocellular carcinoma patients, in 32.7% in intravenous drug users, in 9.7% in prostitutes and in 7% of healthy blood donors (Poovorawan et al, 1998). In the United States, TTV viremia affected 8.4% to 41.6% of volunteer blood donors, 13% of commercial blood donors, 17% of intravenous drug abusers, and 2% of non-A-E hepatitis patients (Desal et al, 1999; Handa et al, 2000).

Despite the ubiquity of TTV in the general population it has recently been noted that TTV remains a virus in search of a disease (Cossart, 2000). TTV has been isolated from patients with non-A-G hepatitis whose virus titers were found to be several logs higher in the liver than in the corresponding sera, suggesting active viral replication in the liver (Okamoto et al, 1999). TTV has also been detected in the bile of five patients with TTV viremia who underwent bile drainage or cholecystectomy for severe cholestasis; in additions, the virus was identified in the feces of one of these patients (Davidson et al, 1999). This evidence suggests that TTV can actively replicate in the human host and spread either by the fecal-oral route or by intravenous inoculation. The role of TTV in producing human disease is yet to be determined: whereas TTV was detected in 21% of healthy children and in 66% of children with chronic hepatitis B or C, no differences in liver enzymes were found in children with chronic hepatitis with TTV infection compared with children with chronic hepatitis without TTV infection. (Gerner, 2000). TTV has been linked as a co-infection with chronic viral hepatitis as well as in healthy controls, with TTV present in 77.8% of hepatitis B virus (HBV) positive patients, in 36.4% with hepatitis C virus (HCV), in 63.6% of non-B, non-C chronic hepatitis and in 59% of normal subjects (Kim et al, 2000; Matsubara, 2000). TTV has also been associated with patients with acute hepatitis: 12.6% in patients with acute hepatitis of defined etiology; 16.6% in patients with non-A-E hepatitis as well as in 6.6% of a healthy control group (Fabris et al, 2000). Though associated with hepatitis of defined and undefined etiology, TTV's role in producing liver injury or damage has not been determined (Cleavinger et al, 2000; Gad et al, 2000; Lefrere et al, 2000).

Current evidence suggests that TTV is widely distributed in the human population, can produce both an acute and chronic infection and is transmitted by transfusion or by fecal-oral routes. It remains to be determined whether TTV is associated with acute or chronic viral hepatitis and whether it is a primary hepatitis virus (Matsumoto et al, 1999). The purpose of this study is to determine the infection prevalence rates of TTV in a population located in Northern Thailand and to determine its significance in patients with non-A to E acute hepatitis.

MATERIALS AND METHODS

Study groups

Serum samples for cases of non-A-E hepatitis and comparison groups were collected from an extensive clinical sera bank from a number of both Thai and United States Army Institutional Review Board and Human Use Committee approved studies. These studies were performed or are currently being conducted by the Department of Virology, United States Army Medical Component- Armed Forces Research Institute of Medical Sciences (USAMC-AFRIMS), Bangkok. All sera samples were stripped of patient identifiers and tested blindly. Adults were defined as those of at least 18 years of age; adolescents were those aged 10-18 years; children were those less than 10 years old.

Sera from adult, adolescent and child patients suffering from non-A-E hepatitis were collected during the years 1995 to 1997 in a large passive hepatitis surveillance project in Kamphaeng Phet Province, Northwestern Thailand, as part of an extended phase III trial of an inactivated hepatitis A vaccine. The hepatitis surveillance program offered free hepatitis diagnostic evaluation to all government and private physicians within Kamphaeng Phet Province for patients with suspected acute viral
hepatitis, excluding neonatal jaundice, alcohol- or drug-induced hepatitis and chronic liver disease. Patients were classified as having non-A-E hepatitis if they had clinical symptoms or signs of an acute illness consistent with hepatitis and a 1.5 times increase in the serum levels of alanine aminotransferase (ALT) and/or aspartate aminotransferase (AST) above the upper limit of normal. In addition, acute serologic markers for hepatitis A, B, C, E, Epstein-Barr virus (EBV), cytomegalovirus (CMV) and leptospirosis were negative.

Sera for comparison groups in adults, adolescents and children were collected during the years 1994 to 2000. The sera for the acute viral hepatitis A or B comparison groups of adults, adolescents and children were collected in Kamphaeng Phet Province as part of the hepatitis surveillance project and in Bangkok and in Nan District, Northern Thailand, during several hepatitis A virus outbreaks. All volunteers were acutely symptomatic and were positive for either hepatitis A virus (HAV) IgM (HAV Ab-m, Abbot Laboratories) or hepatitis B virus (HBV) IgM (Corzyme, Abbot Laboratories). Sera were collected from hospitalized patients with other non-viral hepatitis illnesses in adults, adolescents and children from Kamphaeng Phet Provincial Hospital. All patients had laboratory-confirmed acute illnesses, including dengue, malaria, typhoid fever, viral or bacterial meningitis and scrub typhus.

Specimens from healthy adult subjects were collected from asymptomatic Royal Thai Army military recruits as part of a routine screening program for entrance into military service. Recruits came from all provinces in Thailand and had normal AST, ALT and total bilirubin levels. Specimens from healthy adolescent and child volunteers were collected from Kamphaeng Phet Province as part of a phase IV trial of an inactivated hepatitis A vaccine. All adolescents and children were asymptomatic with normal AST, ALT and total bilirubin and lacked IgM for HAV.

**Serologic tests for causes of hepatitis**

Serology for hepatitis A IgM, hepatitis B IgM and surface antigen, and hepatitis C, was performed using Abbott enzyme immunoassay kits (Abbott Labs Inc). Hepatitis E virus (HEV) total Ig and IgM were determined by using a Walter Reed Army Institute of Research (WRAIR) developed enzyme immunoassay using a recombinant ORF2 HEV protein (Innis et al, in preparation). Leptospirosis IgM, EBV IgM/IgG and CMV IgM/IgG were determined by using PanBio enzyme immunoassay kit (PanBio Inc, Australia).

**Detection of TTV DNA sequence by PCR**

DNA was extracted from sera by the modified method of Okamoto et al (1999). Briefly, 100 μl of sera were mixed with 300 μl of Tris hydrochloride buffer (13.3 mM Tris at pH 8.0) with 6.7 mM EDTA, 0.67% (wt/vol) sodium dodecyl sulfate (SDS) and 133 μg/ml of proteinase K; the mixture was gently vortexed and incubated at 70°C for 3 hours. DNA was extracted by adding 400 μl of phenol-chloroform (1:1) volume with carrier tRNA (10 pg/ml). DNA was precipitated with 3M sodium acetate (pH 5.2, 1/10 vol) and absolute chilled ethanol (2 vol) and incubated at -70°C for 30 minutes. The pellet was washed with 75%-chilled ethanol, dried at room temperature and resuspended in 25 μl of distilled water. TTV DNA was amplified by a semi-nested polymerase chain reaction (PCR) by the method of Naoumov et al (1998). For the first PCR reaction, 10 pmol of primer A (sense primer 5'-ACA GAC AGA GGA GAA GGC AAC ATG-3'), 10 pmol of primer B (antisense primer 5'-CTG GCA TTT TAC CAT TTC CAA AGT T-3') and 10 μl of DNA were used in a 50 μl PCR mixture containing 1 X PCR buffer, 0.2 mM dNTP, 2 mM magnesium chloride and 0.5 U Taq polymerase (PE Applied Biosystems, Branchburg, NJ). PCR amplification was performed using a Perkin Elmer 9600 for 35 cycles: 94°C for 30 seconds, 58°C for 30 seconds, 72°C for 45 seconds followed by 7 minutes at 72°C after the last cycle. The second PCR reaction was performed with primer C (sense primer 5'-GAC AGA GGA GAA GGC AAC ATG T-3') and 10 μl of DNA were used in a 50 μl PCR mixture containing 1 X PCR buffer, 0.2 mM dNTP, 2 mM magnesium chloride and 0.5 U Taq polymerase (PE Applied Biosystems, Branchburg, NJ). PCR amplification was performed using a Perkin Elmer 9600 for 35 cycles: 94°C for 30 seconds, 58°C for 30 seconds, 72°C for 45 seconds followed by 7 minutes at 72°C after the last cycle. The second PCR reaction was performed with primer C (sense primer 5'-GAC AGA GGA GAA GGC AAC ATG T-3') and antisense primer B. 1 μl from the first PCR reaction was transferred to
another tube containing a second PCR mixture and amplified for another 25 cycles at 94°C for 30 seconds, 58°C for 30 seconds, 72°C for 30 seconds with the last cycle followed by 7 minutes at 72°C. The resultant 272 base-pair amplicon was detected on a 2% agarose gel, stained with ethidium bromide and photographed under ultraviolet light.

To confirm that the 272 base-pair amplicon was detecting TTV virus, direct sequencing was performed commercially (Bioservice Unit, Bangkok, Thailand) on the amplicon from 3 positive sera samples from the non-A-E hepatitis group using a 377 DNA automated sequencer (PE Applied Biosystems). Sequences were compared with known TTV sequences to the ORF1 gene in the GenBank with sequence alignment performed using the BLASTN 2.0.10 program (Altschul et al, 1997). All three amplicons were 95% to 98% aligned with known TTV sequences. These amplicons were then used as positive controls. Both positive and negative controls were run simultaneously for all PCR reactions.

Statistical analysis

Statistical analysis was performed using EpiInfo version 6.1 (Centers for Disease Control and Prevention) and SPSS for Windows version 8.0 (SPSS Inc, Chicago, Ill).

RESULTS

Demographics of study groups

Acute hepatitis non-A-E group: A total of 112 subjects were studied in the acute hepatitis non-A-E group. The mean ± standard deviation (SD) age for the entire group was 38 ± 18 years, with 40% females and 60% males. The mean ALT and AST levels were 276 U/l ± 390 U/l and 327 U/l ± 540 U/l respectively. In the adult group (N=99) the mean age was 42 ± 16 years, with 40% females and 60% males. The mean ALT and AST levels were 256 U/l ± 331 U/l and 300 U/l ± 392 U/l respectively. For adolescents (N=6) the mean age was 11 years ± 2 years, with 50% females and 50% males. The mean ALT and AST levels were 160 U/l ± 82 U/l and 209 U/l ± 133 U/l respectively. For children (N=7) the mean age was 6 years ± 2 years, with 29% females and 71% males. The mean ALT and AST levels were 650 U/l ± 919 U/l and 815 U/l ± 1,600 U/l respectively.

Viral hepatitis group: The hepatitis group consisted of 72 patients with either acute hepatitis A or B. The mean age of the hepatitis A or B group was 23 years ± 10 years, with 32% females and 68% males. The mean ALT and AST levels were 282 U/l ± 569 U/l and 219 U/l ± 514 U/l respectively. In the adult group (N=54) the mean age was 27 years ± 9 years, with 39% females and 61% males. The mean ALT and AST levels were 359 U/l ± 640 U/l and 270 U/l ± 586 U/l respectively. For adolescents (N=8) the mean age was 14 years ± 2 years, with 25% females and 75% males. The mean ALT and AST levels were 43 U/l ± 34 U/l and 51 U/l ± 54 U/l respectively. For children (N=10) the mean age was 8 years ± 2 years, with 100% males. The mean ALT and AST levels were 60 U/l ± 31 U/l and 83 U/l ± 70 U/l respectively.

Acute non-hepatitis illnesses: The acute non-hepatitis illness group consisted of 48 patients. The mean age was 20 years ± 12 years, with 35% females and 65% males. The mean ALT and AST levels were 198 U/l ± 868 U/l and 251 U/l ± 1,061 U/l respectively. In the adult group (N=25) the mean age was 29 years ± 8 years, with 36% females and 64% males. The mean ALT and AST levels were 91 U/l ± 153 U/l and 116 U/l ± 181 U/l respectively. For adolescents (N=11), the mean age was 14 years ± 2 years, with 55% females and 45% males. The mean ALT and AST levels were 47 U/l ± 24 U/l and 91 U/l ± 77 U/l respectively. For children (N=12), the mean age was 7 years ± 3 years, with 17% females and 83% males. The mean ALT and AST levels were 560 U/l ± 1,724 U/l and 677 U/l ± 2,115 U/l respectively.
### Table 1
Prevalence and risk of TT virus in non-A to E hepatitis and comparison groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>No positive (%)</th>
<th>Odds ratio (95% CI)*</th>
<th>Significance (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-A to E hepatitis</td>
<td>112</td>
<td>21 (19)</td>
<td>1.6* (0.8, 3.2)</td>
<td>0.014</td>
</tr>
<tr>
<td>Adults</td>
<td>99</td>
<td>16 (16)</td>
<td>1.3b (0.6, 2.8)</td>
<td>0.660</td>
</tr>
<tr>
<td>Adolescents</td>
<td>6</td>
<td>0 (0)</td>
<td>0.0c</td>
<td>1.00</td>
</tr>
<tr>
<td>Children</td>
<td>7</td>
<td>5 (72)</td>
<td>25.8d (2.6, 361.8)</td>
<td>0.001</td>
</tr>
<tr>
<td>Viral hepatitis group</td>
<td>72</td>
<td>12 (17)</td>
<td>1.2e (0.5, 2.7)</td>
<td>0.870</td>
</tr>
<tr>
<td>Adults</td>
<td>54</td>
<td>12 (22)</td>
<td>0.7f (0.3, 1.7)</td>
<td>0.450</td>
</tr>
<tr>
<td>Adolescents</td>
<td>8</td>
<td>0 (0)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Children</td>
<td>10</td>
<td>0 (0)</td>
<td>-</td>
<td>0.003</td>
</tr>
<tr>
<td>Non-hepatitis illnesses</td>
<td>48</td>
<td>8 (17)</td>
<td>1.2h (0.4, 3.1)</td>
<td>0.930</td>
</tr>
<tr>
<td>Adults</td>
<td>25</td>
<td>2 (8)</td>
<td>2.2i (0.4, 15.1)</td>
<td>0.520</td>
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<tr>
<td>Adolescents</td>
<td>11</td>
<td>3 (27)</td>
<td>0.0j (0.0, 4.6)</td>
<td>0.510</td>
</tr>
<tr>
<td>Children</td>
<td>12</td>
<td>3 (25)</td>
<td>7.5k (0.7, 115.5)</td>
<td>0.070</td>
</tr>
<tr>
<td>Healthy subjects</td>
<td>72</td>
<td>4 (6)</td>
<td>3.9l (1.2, 14.2)</td>
<td>0.020</td>
</tr>
<tr>
<td>Adults</td>
<td>50</td>
<td>3 (6)</td>
<td>3.6m (0.7, 13.8)</td>
<td>0.140</td>
</tr>
<tr>
<td>Adolescents</td>
<td>10</td>
<td>1 (10)</td>
<td>0.0n (0.0, 33.6)</td>
<td>1.00</td>
</tr>
<tr>
<td>Children</td>
<td>12</td>
<td>0 (0)</td>
<td>-</td>
<td>0.002</td>
</tr>
</tbody>
</table>

*All odds ratios expressed as odds of TTV in non-A through E group versus comparison groups.

Yates corrected χ², compared with all healthy volunteers, hepatitis group and other illnesses.

Yates corrected χ², compared with all healthy adult volunteers, adult hepatitis group and adult other illnesses.

Fisher’s exact test, compared with all healthy adolescent volunteers, adolescent hepatitis group and adolescent other illnesses.

Fisher’s exact test, compared with all healthy children volunteers, children hepatitis group and children other illnesses.

Yates corrected χ², in comparison to all non-A to E hepatitis.

Yates corrected χ², in comparison to adult non-A to E hepatitis.

Fisher’s exact test, in comparison to children non-A to E hepatitis.

Fisher’s exact test, in comparison to all non-A to E hepatitis.

Fisher’s exact test, in comparison to adolescent non-A to E hepatitis.

Fisher’s exact test, in comparison to children non-A to E hepatitis.

Fisher’s exact test, in comparison to all non-A to E hepatitis.

Fisher’s exact test, in comparison to adult non-A to E hepatitis.

Fisher’s exact test, in comparison to adolescent non-A to E hepatitis.

Yates corrected χ², in comparison to children non-A to E hepatitis.

Healthy subjects: The healthy subjects group consisted of 72 subjects. The mean ALT and AST levels were 20 U/l ± 8 U/l and 35 U/l ± 2 U/l respectively. Healthy adults (N=50), were military recruits who were aged between 18 and 21 years. The exact age identifier was stripped away as per requirements of the Royal Thai Army for sera sample testing. All in this group were males with mean ALT and AST levels of 23 U/l ± 7 U/l and 42 U/l ± 15 U/l respectively. For adolescents (N=10), the mean age was 12 years ± 1 year, with 30% females and 70% males. The mean ALT and AST levels were 13 U/l ± 4 U/l and 10 U/l ± 2 U/l respectively. For children (N=12), the mean age was 7 years ± 1 year, with 33% females and 67% males. The mean ALT and AST levels were 15 U/l ± 5 U/l and 32 U/l ± 5 U/l.
TTVirus and non-A-E hepatitis

Table 2
Presence of TTV and mean alanine aminotransferase (ALT) and mean aspartate aminotransferase (AST) levels (U/l).

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean ALT</th>
<th>Significance*</th>
<th>Mean AST</th>
<th>Significance*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TTV+</td>
<td>TTV-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-A to E hepatitis</td>
<td>395</td>
<td>248</td>
<td>0.122</td>
<td>609</td>
</tr>
<tr>
<td>Adults</td>
<td>246</td>
<td>258</td>
<td>0.876</td>
<td>456</td>
</tr>
<tr>
<td>Adolescents</td>
<td>-</td>
<td>160</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Children</td>
<td>869</td>
<td>104</td>
<td>0.366</td>
<td>1,099</td>
</tr>
<tr>
<td>Viral hepatitis group</td>
<td>291</td>
<td>281</td>
<td>0.952</td>
<td>90</td>
</tr>
<tr>
<td>Adults</td>
<td>291</td>
<td>378</td>
<td>0.627</td>
<td>90</td>
</tr>
<tr>
<td>Adolescents</td>
<td>-</td>
<td>43</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Children</td>
<td>-</td>
<td>60</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Non-hepatitis illness</td>
<td>40</td>
<td>230</td>
<td>0.214</td>
<td>76</td>
</tr>
<tr>
<td>Adults</td>
<td>49</td>
<td>95</td>
<td>0.182</td>
<td>92</td>
</tr>
<tr>
<td>Adolescents</td>
<td>53</td>
<td>45</td>
<td>0.789</td>
<td>114</td>
</tr>
<tr>
<td>Children</td>
<td>20</td>
<td>740</td>
<td>0.309</td>
<td>27</td>
</tr>
<tr>
<td>Healthy volunteers</td>
<td>24</td>
<td>20</td>
<td>0.833</td>
<td>34</td>
</tr>
<tr>
<td>Adults</td>
<td>27</td>
<td>23</td>
<td>0.943</td>
<td>42</td>
</tr>
<tr>
<td>Adolescents</td>
<td>13</td>
<td>13</td>
<td>0.981</td>
<td>8</td>
</tr>
<tr>
<td>Children</td>
<td>-</td>
<td>15</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*Significance determined by Student’s t-test, two-tailed between ALT/AST and TTV positive or negative within each group.

Association of TTV with acute non-A-E hepatitis

The overall prevalence of TTV among all cases and comparison groups was 14.8% (45/304). Prevalence varied by study groups (Table 1), with a TTV prevalence in all non-A-E hepatitis patients of 19%; in healthy volunteers the prevalence was 6%; in the hepatitis group 17% and for the other illnesses 17%. Significant risk of TTV in the non-A-E hepatitis group was seen in children (OR 25.8, p<0.001) and in comparison with healthy volunteers (OR 3.9, p<0.05). Children with non-A-E hepatitis have the highest prevalence for TTV infection with 72% (5/7) positive. In contrast, children hospitalized with other infections had a TTV prevalence of 25% (3/12). TTV was not detectable in healthy children or children with hepatitis A or B.

Presence of TTV and liver enzyme abnormalities

The association between TTV presence and hepatic transaminase levels was determined for each of the study groups. Overall mean ALT for TTV-positive subjects was 271 U/l compared with 193 U/l in TTV negative-subjects (p = 0.33, t-test). For AST, the mean level in TTV positive-subjects was 330 U/l compared with 201 U/l in TTV-negative-subjects (p = 0.29, t-test). Table 2 shows the AST and ALT levels within the study groups with and without the presence of TTV. TTV was associated with higher levels of AST and ALT only for the non-A-E hepatitis group and significant only for AST. Children with non-A-E hepatitis and TTV-positive had an 8-fold higher ALT and a 10-fold higher AST compared with children with non-A-E hepatitis without TTV. These differences were not statistically different. The presence of TTV in healthy volunteers or in HAV or HBV hepatitis was not associated with higher liver enzyme levels.

DISCUSSION

The main observation in this study is the association of TTV viremia with acute non-A-
E hepatitis compared with patients with viral hepatitis or acute non-hepatitis illnesses and healthy subjects. A strong association was observed among children with acute non-A-E hepatitis and TTV compared with children in the comparison groups. Previous studies have also documented high prevalence rates of TTV in children with hepatitis of unknown etiology; however, the TTV prevalence was no different to that of children without hepatitis (Iriyama, 1999). Other studies have demonstrated a higher prevalence of TTV in patients with hepatitis of unknown etiology; however, no link has been established between TTV and hepatocellular injury (Fabris et al, 2000). The other observation in this study is that among subjects with acute non-A-E hepatitis, higher levels of liver enzyme abnormalities (AST) are observed in patients with TTV compared with patients without TTV infection. This observation was most marked in TTV-positive children with non-A-E hepatitis who demonstrated 8- to 10-fold higher levels of ALT and AST respectively compared with children without TTV and non-A-E hepatitis though statistically not significant. Other studies have also documented an association of TTV with an elevation in hepatic transaminase levels though pathological evidence of hepatic injury from this virus has not been documented (Fabris et al, 2000; Rodriguez-Inigo et al, 2000).

What is the significance of TTV infection? It is clear from the literature that TTV is still in search of a disease (Cossart, 2000). Previous studies have demonstrated a wide geographic distribution of TTV and its presence in otherwise healthy individuals. It is apparent that TTV is a real infection and can be transmitted from host to host by body fluids or a fecal-oral route and that infection results in active replication of the virus and, in some individuals, produces a chronic carrier state. Virological evidence of higher titers in the liver or bile compared with serum also suggests active replication in the liver (Okamoto et al, 1999). The presence of TTV in hepatocytes as detected by in situ hybridization has been observed in 17 TTV-viremic patients but in none of those found to be TTV-negative (Rodriguez-Inigo et al, 2000). No histologic changes in the hepatocytes were seen in cells positive for TTV (Rodriguez-Inigo et al, 2000). Previous studies have demonstrated an association of TTV and elevated liver enzymes. In one study, 5% of samples obtained from donors with elevated ALT had TTV DNA detected by PCR compared with 0.7% of those with normal ALT (Cleavinger et al, 2000). In patients with elevated ALT levels, the mean ALT values in patients with TTV was 296 U/I compared with an ALT of 95 U/I in patients without TTV (Cleavinger et al, 2000). The clinical significance of TTV infection was investigated in Egyptian patients with chronic liver disease and volunteer blood donors by a cross-sectional analysis. The prevalence of TTV DNA did not differ among patients with chronic hepatitis B (46%), chronic hepatitis C (31%), or schistosomal liver disease (36%) (Gad et al, 2000). A study of patients having a well-defined date of TTV infection during a mean period of 3.1 years found chronic infection in 85% of cases (Lefrere et al, 2000). TTV carriage appeared clinically benign in all patients without evidence of a disease potentially linked to the TTV infection with the majority of TTV carriers having no biochemical evidence of liver disease (Lefrere et al, 2000). In Taiwan, TTV was found to play an insignificant role in acute fulminant and non-fulminant hepatitis (Huang et al, 2000). TTV infection in Taiwan was more frequent in high-risk groups (26-70%), patients with acute or fulminant non-A-E hepatitis (42-45%), and hepatitis C carriers (36%) than in healthy adults (10%) and hepatitis B carriers (15%) (Kao et al, 1999). TTV infection was not associated with hepatitis or an increase in liver enzyme abnormalities (Kao et al, 1999).

One study of TTV in non-human primates provides compelling evidence that infection can occur after intravenous or oral administration and results in active viral replication and persistent infection (Luo et al, 2000). Rhesus monkeys became viremic 4-7 days after parental administration and 7-10 days after oral administration. TTV was detected in the feces, bile and hepatocytes. A prolonged carrier state
developed with persistent viremia and virus excretion in feces for more than 6 months. There was no live enzyme or pathological evidence of hepatocellular damage.

Our current findings suggest a role for TTV in acute non-A-E hepatitis with an associated increase in liver enzyme abnormalities, especially in children. TTV has been previously detected in children with 20% prevalence in those with hepatitis of unknown etiology and 23% in healthy children (Iriyama et al, 1999). Children who have received transfusions or are hemophiliacs have a higher prevalence (50%). TTV prevalence has been found to be age-dependent with TTV detected in 10% of the infants under 6 months old and 20% of children aged 7 to 12 months. Similar findings were noted in a study conducted in Rio de Janeiro, where TTV prevalence increased with age from 17% among children under the age of 11 years to 57% in people older than 50 years (Saback et al, 1999). As in other hepatic viral illnesses, children may show TTV infection in a way that differs from that of adults. Perhaps children, during their first TTV infection, suffer clinical hepatitis from which a proportion go on to develop a benign chronic carrier state. The significance of these findings remains to be determined and further studies are needed to clarify the role of TTV as a human pathogen.

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