Molecular Detection of Transfusion Transmitted Virus Coinfection With Some Hepatotropic Viruses

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Context.—A novel human DNA virus was isolated from the serum of a patient with posttransfusion hepatitis; it was named transfusion transmitted virus (TTV).

Objective.—To ascertain the influence of TTV (detected by polymerase chain reaction amplification of a conserved region of the viral genome) coinfection in individuals infected with hepatitis viruses (A, B, and C) and to investigate the putative role played by TTV in hepatic dysfunction in individuals with acute non-A-E hepatitis.

Design.—Sixty-two patients with viral hepatitis were included in the study in addition to 18 blood donors. Viral study of 4 hepatotropic viruses (A, B, C, and E) was carried out. Study for TTV DNA was performed by nested polymerase chain reaction.

Results.—The prevalence of TTV was not statistically different between hepatitis patients and blood donors, and it was not correlated to the levels of the hepatic aspartate aminotransferase and alanine aminotransferase between individuals evidencing dual infection with hepatitis B and C viruses and healthy blood donors. However, in the group of patients with viral hepatitis of unknown etiology (non-A-E), those evidencing TTV viremia had statistically significant lower levels of alanine aminotransferase (P = .03) and aspartate aminotransferase (P = .04) than those who were TTV negative.

Conclusions.—We can conclude that TTV is a frequent virus isolated from patients with various types of viral hepatitis, from cases of hepatitis without obvious viral agent, and from the healthy population. TTV has no effect on biochemical markers of associated viral hepatitis. It may be associated with a mild form of non-A-E hepatitis.

(Arch Pathol Lab Med. 2006;130:1680–1683)

MATERIALS AND METHODS

This study included 62 patients with viral hepatitis. Patients were attending Mansoura University Hospital, Egypt. There were 8 patients with hepatitis A, 15 patients with hepatitis B, 19 patients with hepatitis C, 5 patients with combined hepatitis B and C, and 15 patients with non-A through E acute hepatitis. Eighteen blood donors were also included in the study with matched age and sex. The studied subjects were 57 men and 23 women with mean ± SD age 42.2 ± 8.1 years. Informed written consent was obtained from all participants and the Ethics Committee of the Mansoura University Hospital approved the study.

Each subject included in the study was subjected to full medical history and thorough clinical examination. Laboratory investigations were performed including liver function tests by autoanalyzer (Express II; Bayer, Tarrytown, NY).
DETECTION OF HEPATOTROPIC VIRUSES (A, B, C, E, AND TTV)

Complete serologic profiles for hepatitis viruses A, B, C, and E were evaluated. Immunoassay was carried out (Equipar-Italy, Saronna, VA) for hepatitis A immunoglobulin (Ig) M, hepatitis B surface antigen, IgG for hepatitis C, and IgM for hepatitis E.

Detection of TTV was performed by nested PCR with a commercially available kit for extraction of viral DNA from the serum (Omega Biotek, Bolton, England) and pure Tag ready to go PCR beads for amplification (Amersham Bioscience, Buckinghamshire, England). DNA was first extracted from the serum sample followed by amplification by nested PCR with TTV specific primers according to manufacturer instructions. The target sequence from the ORF 1 region of TTV genome was used for nested PCR. For the first round, sense primer (5’-CAGACAGAGGGAG AAGACATG-3’) and antisense primer (5’-TACCTTTTAG CTCTCTCTTA-3’) were used. For the second round, sense primer (5’-GGMAATGTGTTTGGATACCTGG-3’) and antisense primer (5’-CCCTCCTGGCATTTT CCA-3’) were used. The amplified DNA products were analyzed by agarose gel electrophoresis. A band of 227 base pairs in length was considered positive for TTV DNA. Nonspecific amplification product is prevented by strict PCR conditions. Sterile distilled water was used as negative control. A reference-sizing ladder of known fragment lengths of control DNA was run.

STATISTICAL ANALYSIS

Values are given as means ± SD or as the number of subjects and proportions. One-way analysis of variance test and independent samples Student t test were used for group comparisons of normally distributed variables, and the Kruskal-Wallis test and Mann-Whitney U test were used for comparisons of variables with skewed distribution. The chi-square test was used to compare proportions.

RESULTS

Sixty-two patients with viral hepatitis were included in the study in addition to 18 blood donors. Viral study of 4 hepatotropic viruses (A, B, C, and E) was carried out. Study for TTV DNA was performed by nested PCR.

TTV DNA was detected with high percentage among patients with combined hepatitis B and C (80%) followed by patients with hepatitis C (78.9%) and hepatitis B virus (66.7%). The lowest rate for positive cases was among patients with hepatitis A (37.5%). The detection rate among patients with non-A through E hepatitis was 40%. Among blood donors the positive rate was 61.1% (Table 1).

TTV was not correlated to the levels of the hepatic aspartate aminotransferase (AST) and alanine aminotransferase (ALT) between individuals evidencing dual infection with hepatitis B and C viruses and healthy blood donors. However, in the group of patients with viral hepatitis of unknown etiology (non-A through E), those evidencing TTV viremia had statistically significant lower levels of ALT and AST than those who were TTV negative (P = .03, P = .04 respectively, Table 2).

The Figure demonstrates DNA marker and positive samples for TTV by PCR.

The level of hepatic enzymes ALT and AST was statistically lower in hepatitis patients coinfected with TTV compared with those who were TTV negative (P = .005, P = .001, respectively). Also, the level of ALT had a sta-
trast, several series of hepatitis C virus patients’ coinfection with TTV appeared to be associated with increased severity of biochemical and histologic parameters of liver damage.22,23 Also, Kasirag et al24 found that children coinfected with hepatitis B virus and TTV had evidence of greater liver damage.

Interestingly, in patients with acute hepatitis with non-A-E liver enzymes had statistically significant lower values in patients with TTV viremia. There was debate regarding levels of liver enzymes in those patients as some authors found that TTV viremic patients had significantly higher levels than non-PTT viremic patients.25,26 Other authors did not find significant difference in serum ALT or direct bilirubin between TTV viremic and nonviremic patients.19,27 Liver damage associated with TTV might become evident only when the extent of virus replication is more than a certain threshold.28 So, it could be speculated that TTV was associated with a mild form of hepatic infection in these patients.

Patients with dual hepatitis C virus and TTV had statistically significant lower level of AST than patients who were negative for TTV. Also, the level of hepatic enzymes ALT and AST was statistically lower in hepatitis patientscoinfected with TTV compared with those who were TTV negative. It is hypothesized that TTV could be locally or systemically immunosuppressive by replicating in polyclonal stimulated, but not in resting, human peripheral blood mononuclear cells and possibly dying as a result.29 Thus, TTV coinfection with hepatitis viruses could lead to downregulation of immune-mediated damage to infected liver cells and reduce the level of the released liver enzymes.

From this study, it can be concluded that TTV is a frequent virus isolated from patients with various types of viral hepatitis, in cases of hepatitis without obvious viral agent, and from the healthy population. TTV has no effect on biochemical markers of associated viral hepatitis. It is associated with a mild form of non-A through E hepatitis.

Table 3. Liver Function Tests in the Studied Subjects According to Presence of Transfusion Transmitted Virus (TTV) Viremia*

<table>
<thead>
<tr>
<th></th>
<th>TTV Negative (n = 31)</th>
<th>TTV Positive (n = 17)</th>
<th>TTV and HCV Positive (n = 15)</th>
<th>TTV and HBV Positive (n = 10)</th>
<th>TTV and HAV Positive (n = 3)</th>
<th>TTV and HBV and HCV Positive (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin Mean ± SD</td>
<td>4.89 ± 0.52</td>
<td>5.1 ± 0.2</td>
<td>4.9 ± 0.04</td>
<td>4.7 ± 0.52</td>
<td>5.2 ± 0.12</td>
<td>4.9 ± 0.5</td>
</tr>
<tr>
<td>P</td>
<td>.12</td>
<td>.12</td>
<td>.13</td>
<td>.19</td>
<td>.88</td>
<td></td>
</tr>
<tr>
<td>Total bilirubin Mean ± SD</td>
<td>3.5 ± 0.7</td>
<td>1.9 ± 0.5</td>
<td>2.4 ± 0.2</td>
<td>6.5 ± 2.1</td>
<td>3.3 ± 0.5</td>
<td>4.6 ± 1.6</td>
</tr>
<tr>
<td>P</td>
<td>.15</td>
<td>.15</td>
<td>.34</td>
<td>.03</td>
<td>.91</td>
<td>.59</td>
</tr>
<tr>
<td>Alanine aminotransferase Mean ± SD</td>
<td>154.1 ± 26</td>
<td>39.1 ± 10.04</td>
<td>70.3 ± 3.6</td>
<td>215.7 ± 80</td>
<td>101.7 ± 18.3</td>
<td>93.2 ± 10.4</td>
</tr>
<tr>
<td>P</td>
<td>.005</td>
<td>.04</td>
<td>.04</td>
<td>.19</td>
<td>.51</td>
<td>.38</td>
</tr>
<tr>
<td>Aspartate aminotransferase Mean ± SD</td>
<td>124.8 ± 16</td>
<td>52.1 ± 14.2</td>
<td>59 ± 4.9</td>
<td>109.2 ± 17</td>
<td>150 ± 50</td>
<td>142.8 ± 27</td>
</tr>
<tr>
<td>P</td>
<td>.01</td>
<td>.003</td>
<td>.03</td>
<td>.53</td>
<td>.54</td>
<td>.62</td>
</tr>
</tbody>
</table>

* HCV indicates hepatitis C virus; HBV, hepatitis B virus; and HAV, hepatitis A virus.

Comment

TTV DNA has been reported in patients with a broad spectrum of hepatic disorders as well as in healthy people.8

The percentage of TTV coinfection with hepatitis B, C, and A in our study was 66.7%, 78.9%, and 37.5%, respectively. These results were comparable to those reported in previous studies ranging from 20% to 75.7% for hepatitis C9-11 and from 40% to 75% for hepatitis B patients.9,10,12 Similar rate of infection among hepatitis A patients was reported by He et al13 (20% [6/30]). Nevertheless, in United Arab Emirates the rates were higher, 97.9% and 95.7% among hepatitis A patients with hepatitis B virus or hepatitis C virus, respectively.14

The prevalence of TTV viremia among healthy blood donors was 61.1%. There was statistically insignificant difference between prevalence of TTV among hepatitis patients and blood donors. This finding clarifies the remarkable feature of TTV with its extraordinarily high prevalence of chronic viremia in apparently healthy people up to nearly 100% in some countries15; it was 80% in Egypt in a previous study.16 Similarly Lyra et al17 found the same prevalence of TTV among patients with hepatitis and normal controls.

Whether TTV was responsible for non-A through E acute hepatitis or not was investigated. Among 15 patients with acute hepatitis 6 patients (40%) had TTV viremia. In hepatitis of unknown etiology, TTV infection was observed in 26% to 71% of cases.18

Surprisingly, the prevalence of TTV was not statistically different between hepatitis patients and blood donors. Because of its high prevalence in the human population, young children included, it is possible that TTV may be mainly transmitted by the oral fecal route or by saliva droplets.19

TTV viremia was not correlated to the levels of the hepatic AST and ALT between individuals evidencing dual infection with hepatitis B and C viruses and healthy blood donors. Similar results were found by Zhao et al,20 Moriyama et al,21 and Chattopadhyay et al22 who reported that coinfection with TTV did not modify the serology or the biochemical markers of chronic hepatitis B or C. In contrast, several series of hepatitis C virus patients’ coinfection with TTV appeared to be associated with increased severity of biochemical and histologic parameters of liver damage.22,23 Also, Kasirag et al24 found that children coinfected with hepatitis B virus and TTV had evidence of greater liver damage.

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References


