RAPID COMMUNICATION

Serum Hepatitis C Virus Sequences in Posttransfusion Non-A, Non-B Hepatitis

By Motohiro Shibata, Tsuneo Morishima, Toyoichiro Kudo, Takako Maki, Shigeo Maki, and Yoshiyuki Nagai

We investigated 17 patients (12 males and 5 females, ages 2 to 57 years old) with posttransfusion non-A, non-B hepatitis to determine relationships between clinical courses and hepatitis C virus (HCV) markers. The patients were grouped according to time course of abnormal serum alanine aminotransferase (ALT) levels into three categories (chronic biochemical disease, biochemically resolved chronic disease, and acute disease). Latest serum samples (1.3 to 10.8 years after blood transfusion) were used to detect antibodies against C100-3 antigen (anti-HCV) by enzyme-linked immunosorbent assay and HCV sequences by polymerase chain reaction (PCR) assay. Of the 17 patients, 13 patients (76.5%) were anti-HCV positive and 8 patients (47.1%), including one anti-HCV negative case, were positive for HCV RNA. In total, 14 patients (82.4%) were positive for either HCV markers.

With respect to clinical course, HCV RNA was detected in six of eight patients (75%) with chronic biochemical disease, and in two of five patients (40%) with biochemically resolved chronic disease. HCV RNA was not detectable in convalescent sera from four patients with acute disease. These results show that there is a relationship between clinical status and HCV viremia, but that normal liver function tests do not always represent the clearance of the virus. Viremia in two patients with normal ALT level suggests that hepatitis is not only caused by viral cytopathic effects, but also by immunologic reactions against virus-infected cells. Thus, PCR is useful in determining the persistence of HCV infection as well as to diagnose anti-HCV negative HCV infection.

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Recent findings. A cDNA from a small, positive-strand RNA virus, designated hepatitis C virus (HCV), was cloned,1 and diagnostic tests to detect antibodies against the recombinant C100-3 antigen (anti-HCV) have shown that anti-HCV was positive in about 80% of patients with posttransfusion non-A, non-B hepatitis (NANBH) and in 50% of patients with sporadic NANBH.2 In addition, recipients of anti-HCV negative blood products were 20 times more likely to have posttransfusion NANBH than recipients of anti-HCV negative blood.3 These results suggest that serum anti-HCV is one of the important markers for infectivity. Furthermore, the polymerase chain reaction (PCR) technique has enabled investigators to detect HCV sequences in liver tissues and sera from patients with NANBH as well as in sera from implicated blood donors.4 These results provide direct evidence that HCV is the major causative agent of NANBH.

A long-term observation of posttransfusion NANBH showed that more than one-half of patients develop chronic liver disease.5 Anti-HCV persisted for more than 6 years in patients with chronic liver disease, while it disappeared in a mean of 4 years in patients with acute liver disease that resolved within the first year.6 However, much less is known about the relation between clinical course and serum HCV sequences, a direct marker of viremia. In this study, we have measured HCV RNA and anti-HCV in sera from patients with different courses of posttransfusion NANBH and show a correlation between abnormal liver function and viremia.

MATERIALS AND METHODS

Patients. Seventeen patients (12 male, 5 female, aged 2 to 57 years) with posttransfusion NANBH were analyzed in this study. The patients had been followed-up from the onset of hepatitis until the present time. They had no clinical or biochemical evidence of pre-existing liver disease and had not received any transfusions in the previous 6 months. They received blood transfusions for open heart surgery (14 patients), anemia caused by blood disease (2 patients) or brain surgery (1 patient) at Meijo hospital (Nagoya, Japan) from 1978 to 1988. Posttransfusion NANBH was defined as two or more consecutive, and otherwise unexplained, elevations in the serum alanine aminotransferase (ALT) that occurred in the 6-month period after the receipt of blood. At least one of these elevations had to be more than twofold the upper limit of normal (normal ALT value is less than 30 U/L). No serologic markers for hepatitis A and B virus infections were detectable. The patients were examined for their liver function weekly or biweekly during acute phase of hepatitis, and thereafter the interval between liver function tests depended on their clinical course. Patients with elevated ALT value were examined monthly or bimonthly, and patients with normal ALT value were examined once or twice yearly.

For the purpose of this report, clinical courses of posttransfusion NANBH were grouped on purely biochemical grounds into three categories in advance. When the disease lasted longer than 6 months and elevation of serum ALT was still observed in the previous year, it was defined as "chronic biochemical disease" (group A).7 No implication will necessarily exist concerning prognosis of group A patients with chronic biochemical disease. When serum ALT was elevated for more than 6 months but recent biochemical examinations have been normal for at least 1 year (on at least three consecutive determinations), the disease was defined as "biochemically resolved chronic disease" (group B). The term "acute" (group C) was used when elevated ALT levels returned to normal range within 6 months from the onset of the disease, and thereafter ALT levels remained normal.

Sera obtained at the latest biochemical examinations were tested for HCV RNA and anti-HCV. In addition, sera from six patients who received blood transfusion but did not develop hepatitis and from 16 healthy individuals without blood transfusion were included in the examinations as controls.

From the Department of Pediatrics, and Institute for Disease Mechanism and Control, Nagoya University School of Medicine, and Department of Pediatric Cardiology and Heart Surgery, Meijo Hospital, Nagoya, Japan.


Address reprint requests to Motohiro Shibata, MD, Department of Pediatrics, Nagoya University School of Medicine, Tsuruma cho 65, Showa ku, Nagoya 466, Japan.

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PCR. Two sets of oligonucleotide primers were synthesized for PCR. One pair of primers was to amplify a 458-bp sequence from the putative nonstructural region 3 of HCV RNA. A sense primer, HCP10, was 5'-TGTACACTCCAGGTTCGTTACAGC-3', and an antisense primer, HCP11, was 5'-ACTTCCACATTTCGATCCACGGGATAGG-3'. An internal probe, HCPNS3, was 5'-TGAAGCTCATACCAAGCAGCAG-3'. The other pair of PCR primers were derived from a 5'-terminal noncoding region of HCV RNA cloned in Japan to amplify 5'-terminal noncoding region. An internal probe, HCP20, was 5'-AAGCACCCTATAAGGTACCCGCTGCA-3'.

RNA was prepared from 100 µL serum by treating in GIT buffer (4.0 mol/L guanidium thiocyanate, 0.1 mol/L Tris-HCl [pH 7.5], 1% 2-mercaptoethanol) and 0.5% sodium lauryl sarcosinate, followed by centrifugation through 5.7 mol/L CsCl/0.01 mol/L EDTA and 0.1% sodium dodecyl sulfate (SDS) at 45°C. The blots were then blotted onto Nytran (Schleicher & Schuell, Germany), and the blots were hybridized to an internal probe end-labeled with γ-32P-adenosine triphosphate (ATP) containing 3X Denhardt's solution, 100 µg/mL salmon sperm DNA, and 0.1% sodium dodecyl sulfate (SDS) at 45°C. The gels were run and analyzed in a blinded fashion. The sensitivity of the PCR assay was determined by analyzing an infectious human serum with HCV titers of approximately 10⁴ chimpanzee infectious doses (CID)/mL, kindly provided by the Association of Non-A, Non-B Hepatitis Research in Japan, Ministry of Welfare, Japan. Serial dilutions were prepared and assayed by PCR and Southern blot hybridization. Both primer sets (HCP10/11 and HCP14/15) could detect approximately 1 CID of the virus.

To avoid false positives with PCR, we applied the contamination prevention measures of Kwok and Higuchi. All PCR and Southern blots were run and analyzed in a blinded fashion.

Anti-HCV assay. Antibodies against C100-3 antigen were measured with the Ortho anti-C100-3 enzyme-linked immunosorbent assay (ELISA; Ortho, Raritan, NJ).

Statistical analysis. The differences between dichotomous variables were analyzed by Fisher's exact test.

RESULTS

Serum was obtained from 17 patients with posttransfusion NANBH at latest biochemical examinations (1.3 to 10.8 years after blood transfusion) and examined for anti-HCV by ELISA and HCV RNA by PCR. Table 1

Table 1. Summary of Patients Grouped According to Clinical Course

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Years After Blood Transfusion</th>
<th>ALT (IU/L)</th>
<th>HCV RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Peak</td>
<td>Latest</td>
</tr>
<tr>
<td>Group A (NANBH with chronic biochemical disease)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. (13/M)</td>
<td>3.0</td>
<td>794</td>
<td>47</td>
</tr>
<tr>
<td>2. (57/M)</td>
<td>1.9</td>
<td>76</td>
<td>40</td>
</tr>
<tr>
<td>3. (10/F)</td>
<td>1.9</td>
<td>391</td>
<td>48</td>
</tr>
<tr>
<td>4. (11/M)</td>
<td>3.8</td>
<td>1310</td>
<td>250</td>
</tr>
<tr>
<td>5. (6/M)</td>
<td>2.4</td>
<td>1626</td>
<td>54</td>
</tr>
<tr>
<td>6. (11/M)</td>
<td>6.5</td>
<td>399</td>
<td>33</td>
</tr>
<tr>
<td>7. (2/M)</td>
<td>2.2</td>
<td>1224</td>
<td>19</td>
</tr>
<tr>
<td>8. (11/M)</td>
<td>12.4</td>
<td>1247</td>
<td>937</td>
</tr>
<tr>
<td>Group B (NANBH with biochemically resolved chronic disease)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>9. (16/M)</td>
<td>10.6</td>
<td>130</td>
<td>9</td>
</tr>
<tr>
<td>10. (56/F)</td>
<td>4.8</td>
<td>282</td>
<td>10</td>
</tr>
<tr>
<td>11. (9/F)</td>
<td>4.9</td>
<td>785</td>
<td>12</td>
</tr>
<tr>
<td>12. (14/M)</td>
<td>9.5</td>
<td>196</td>
<td>7</td>
</tr>
<tr>
<td>13. (11/F)</td>
<td>7.7</td>
<td>891</td>
<td>14</td>
</tr>
<tr>
<td>Group C (NANBH with acute disease)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14. (7/M)</td>
<td>1.8</td>
<td>784</td>
<td>9</td>
</tr>
<tr>
<td>15. (6/M)</td>
<td>1.5</td>
<td>934</td>
<td>22</td>
</tr>
<tr>
<td>16. (14/M)</td>
<td>2.2</td>
<td>487</td>
<td>10</td>
</tr>
<tr>
<td>17. (13/F)</td>
<td>3.8</td>
<td>373</td>
<td>13</td>
</tr>
</tbody>
</table>

*HCP10/11: PCR to amplify NS3 region.
†HCP14/15: PCR to amplify 5'-terminal noncoding region.
showed the summary of the results of ELISA and PCR assays together with clinical data. The 17 patients were grouped according to their clinical courses into three categories as defined in Materials and Methods. Anti-HCV was positive in most patients of group A and B (Table 1), and a total of 13 patients (76.5%) had anti-HCV (Table 2). HCV RNA was amplified with two sets of primers from NS3 region (HCp10/11) or 5′-terminal sequences (HCp14/15). Representative results of PCR are shown in Fig 1. The DNA products had an expected size of 458 bp (HCp10/11) or 298 bp (HCp14/15), and hybridized with the internal probes.

The two primer sets worked equally in detecting the viral genome with one exception (case 6), and a total of eight patients (47.1%) were positive for HCV RNA. With respect to clinical course, HCV RNA was detected in six of eight (75%) patients with chronic biochemical disease (group A), and two of five (40%) patients with biochemically resolved chronic disease (group B). It has to be noted that a single case (no. 8) was positive for HCV RNA even though anti-HCV was negative. In contrast, all four patients with acute disease (group C) were negative for HCV RNA in the convalescent sera.

In total, 14 patients (82.4%) were positive for either HCV markers. Six patients who received blood transfusions but did not develop hepatitis and 16 healthy individuals were negative for both HCV RNA and anti-HCV (Table 2).

DISCUSSION

In this study, we examined sera for HCV RNA and anti-HCV, and found that 82.4% of patients with posttransfusion NANBH were positive for either HCV markers. These results confirm that HCV is the major causative agent for posttransfusion NANBH.1

HCV has considerable variations in nucleotide sequences.10,11 These variations could cause false-negative results by PCR. To minimize false-negative results, we used two different sets of primers for PCR. A single case (no. 6), which is positive only by one of the two primer sets, appears to reflect such sequence variability. However, the fact that the two primer sets gave the same results in the other cases suggests that the regions corresponding to the primer sequences could be conservative.

Van der Poel et al9 reported that several cofactors in anti-HCV positive blood products indicate a high risk of HCV infectivity. A raised serum ALT was a highly significant cofactor for recipient HCV infection, compared with a normal serum ALT (86% v 11%). Therefore, in this study, patients with NANBH were grouped by time course of abnormal serum ALT levels into three categories. Serum HCV RNA was detected in 75% of the group A patients with chronic biochemical disease, 40% of the group B patients with biochemically resolved chronic disease, and none of the group C patients with acute disease. These results show that there is a relation between abnormal liver function and HCV viremia. However, it should be noted that HCV RNA was positive in two patients with biochemically resolved chronic disease. Especially noteworthy, patient no. 9 was HCV RNA positive despite normal biochemical examinations for more than 8 years. This case should be carefully followed-up for exacerbation of liver function. Alternatively, there might be asymptomatic HCV carriers, as suggested by the presence of normal blood donors who transmit HCV hepatitis to recipients.

There is a long delay (mean, 22 weeks) between infection and anti-HCV seroconversion,4 and, therefore, PCR is particularly useful for diagnosing HCV infection during the acute phase.14 In some patients with chronic HCV infection the delay of anti-HCV seroconversion seems to be longer, or anti-HCV may disappear soon.8 Weiner et al8 reported HCV RNA could be detected in liver from anti-HCV negative patients with chronic NANBH. In this study, we also found an anti-HCV negative case who was positive for serum HCV genome. Thus, the absence of anti-HCV does not always exclude HCV infections in chronic NANBH, and PCR is useful for diagnosing such cases.

In conclusion, analysis of patients with posttransfusion NANBH for their clinical course and serum HCV RNA showed that present or recent elevation of serum ALT is likely to be associated with viremia. However, normal liver function does not always represent the clearance of the virus because HCV RNA was also detected in some patients with biochemically resolved chronic disease. These results suggest that hepatitis is not only caused through injury of hepatic cells by viral cytopathic effects, but also by immunologic reactions against virus-infected cells, as in the case of hepatitis B virus infection.15

REFERENCES


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M Shibata, T Morishima, T Kudo, T Maki, S Maki and Y Nagai