ORIGINAL CONTRIBUTION

Long Term Survey of Hepatitis C Virus Infection in Hemodialysis Units in Fukuoka, Japan

Kouzaburo Yamaji¹, Jun Hayashi¹, Yasunobu Kawakami¹, Eriko Yoshimura¹, Yasuhiro Kishihara¹, Misako Ohmiya¹, Yoshitaka Etoh², and Seizaburo Kashiwagi¹

To examine prevalence of hepatitis C virus (HCV) infection and liver dysfunction in hemodialysis units, we surveyed markers for HCV infection and serum alanine aminotransferase (ALT) in hemodialysis patients. 204 hemodialysis patients (111 men and 93 women; mean age, 53 ± 12 years) in four hemodialysis units in Fukuoka, Japan were investigated. All serum samples were tested for antibody to HCV (anti-HCV) by second-generation enzyme-linked immunosorbent assay (ELISA). HCV RNA was detected to identify present HCV infection in the anti-HCV-positive patients by polymerase chain reaction (PCR) using primers deduced from the 5'-noncoding region. Liver dysfunction was defined as an elevated concentration of serum ALT (above 36 IU/liter) tested by a multiple autoanalyser. 105 patients (51.5 percent) were initially positive for anti-HCV, 95 (90.5 percent) of whom were also positive for HCV RNA. Ten became positive for anti-HCV in hemodialysis units during the observation, eight (80 percent) of whom had sustained HCV viremia. The route of transmission of HCV was not clear, but two of these patients had received blood transfusions. Of 95 patients with HCV viremia, 43 (45.3 percent) had had liver dysfunction at least once. In conclusion, HCV infection continues to occur in hemodialysis units not through blood transfusion and many of them become HCV carriers. Liver dysfunction was found in about a half of HCV-infected hemodialysis patients during the observation. J Epidemiol, 1996; 6: 166-171.

HCV, hemodialysis, PCR, liver dysfunction, cohort study

Recent technological advances have greatly improved the prognosis, and quality of life of patients in end-stage renal failure. However, with the advance of science, unknown risk factors continue to become apparent. The prevalence of HCV infection in hemodialysis patients is exceptionally high¹-⁴. In earlier work, we found that 30-50 percent of the hemodialysis patients in Fukuoka, Japan were positive for antibody to HCV (anti-HCV), and that the correlation between the prevalence of anti-HCV and the number of blood transfusions was positive⁵. The prevalence of anti-HCV was significantly higher in hemodialysis patients without episodes of blood transfusion than in blood donors, and increases were seen with the duration of hemodialysis. 86.4 percent of our anti-HCV-positive hemodialysis patients carried HCV RNA in their sera, and 32.7 percent of those with present HCV infection had liver dysfunction⁶.

The exact route of HCV infection in hemodialysis patients is unclear and the prognosis of hemodialysis patients with HCV infection is not well understood. We report here our findings in a 5-year follow-up of patients with HCV viremia, examining HCV markers and serum aminotransferase (ALT).

MATERIALS AND METHODS

Study Population
We surveyed 204 of end-stage renal failure patients (men 111, women 93; mean age 53 ± 12) received maintenance hemodialysis, prospectively. The patients were treated in 4 hemodialysis units in different hospitals located in Fukuoka prefecture, Japan. All of patients underwent blood screening.
for HCV markers from 1989 to 1994. Blood samples were examined at least three times over the 5 years (samples were taken in 1989, 1992, 1993 and 1994). Blood samples were taken by venipuncture using individual, disposable needles, and serum samples were stored at -20 °C until tested. No patient had been treated with interferon for HCV and vaccinated against HBV.

**Serological Testing**

All 204 serum samples were tested for anti-HCV (pHCV-34, pHCV-31, c100) (HCV EIA II, Abbott Laboratories, North Chicago, IL, U.S.A.) by enzyme-linked immunosorbant assay (ELISA)⁹. The manufacturer's instructions were followed throughout. All initially reactive specimens were retested in duplicate. Neutralization tests were done to confirm positivity of the anti-HCV test results. Neutralization by one antigen indicated antibody to the neutralizing antigen. Reactivity of the antibody was determined by adsorbing specimens with purified antigen before the testing the adsorbed and unadsorbed samples for antibody by means of commercially devised ELISA procedures. A 50 percent reduction in counts of the adsorbed samples was regarded as indicating the presence of antibodies.

Hepatitis B surface antigen (HBs Ag), hepatitis B e antigen (HBe Ag) and antibody to HBe Ag (anti-HBe) were measured by radioimmunoassay (AUSRIA II and HBeRIA: Abbott Laboratories, North Chicago, IL, U.S.A.).

To identify the anti-HCV-positive patients presently infected with HCV, serum samples were tested for HCV RNA by nested PCR. RNA was extracted with acid guanidinium thiocyanate-phenol-chloroform. Primers and a probe were constructed for the 5'-noncoding region (where the base sequence is highly conserved) based on sequence data for the HCV⁷. The outer primers used were 5'-CTTGGAGGAACTACTGTCTT-3' (sense) and 5'-AACACTACTCGGCTAGCAGT-3' (antisense). The cDNA was amplified by 25 cycles of PCR. One portion of the products of PCR was sampled, another pair of primers inside the ones described above was designed, and another 30 cycles of the PCR were run. The sense primer used was 5'-TTCACGCAGAAAGCGTCTAG-3', and the antisense primer used was 5'-GTTGATCCAAGAAAGGACCC-3'. Sizes of the PCR products estimated on gel electrophoresis were as predicted; 221 bp by the first stage of PCR and 145 by the second stage of PCR. After 20 minutes, the PCR was repeated, using the same serum samples. In each PCR, we included three negative controls and three positive controls were included. This approach made feasible evaluation of the reproducibility and possible false-negative results caused by untoward events related to the PCR.

Conventional liver function tests were done using a multiple autoanalyzer. Liver dysfunction was defined as an elevated concentration of serum ALT (above 36 IU/liter).

**Statistical Analysis**

$\chi^2$ test was used and, a P value of less than 0.05 was considered to have statistical significance.

**RESULTS**

In 1989, anti-HCV was detected in 105 (51.5 percent) of 204 hemodialysis patients in 4 different units. There were no significant differences in the prevalence of anti-HCV between the sexes (P > 0.05) and the 4 hemodialysis units (P > 0.05) studied (Table 1). HCV RNA was detected in 95 (90.5 percent) of 105 anti-HCV-positive patients. Here too, there were no differences between the sexes.

Changes in anti-HCV over the 5 year period from 1989 to 1994 are shown in Table 2. In none of the 105 patients who were initially positive for anti-HCV, was anti-HCV eliminated from the serum during the observation period. Eleven (11.1 percent) of the 99 patients who were initially negative for anti-HCV seroconverted to positive.

The course of the 11 seroconverted patients is shown in Table 3. HCV RNA was tested in all the serum samples taken during the observation period and was detected in nine patients. In cases 6, 7 and 9, HCV RNA was detected even before anti-HCV was evident in the serum. The case 9 patient had already been positive for HCV RNA in 1989. The case 8 patient received a blood transfusion after mass bleeding due to AV anastomosis in 1993 as did case 10 patient after kidney transplantation in 1991. None of the remaining nine patients received blood transfusion during the observation period. In the remaining two patients (cases 5 and 11), HCV RNA was never positive in the serum. In the case 9 patient, HCV RNA was detected in 1989, 1992 and 1993, but not in 1994.

Of the 193 patients, except for the 11 anti-HCV-seroconverted patients, 56 (29.0 percent) had one or more episodes of elevation in serum ALT level during the observation period. Anti-HCV and HBs Ag in these 56 patients is shown in Table 4. Concordance of anti-HCV and HBs Ag was found in three (5.3 percent) but they were negative for HBe Ag. Anti-HCV alone was found in 43 (76.8 percent). HBs Ag alone was found in one (1.8 percent) and he was also positive for HBe Ag.

The number of anti-HCV positive was significantly more than that of anti-HCV negative among hemodialysis patients with liver dysfunction (P < 0.001). There were no differences between HBs antigen positive and negative patients (P > 0.05).

Liver function tests of 95 patients with HCV viremia were continued for 5 years. Patients with elevated ALT levels in 1989 numbered 14 (14.7 percent) and patients who had elevated ALT levels at least once in the 5-year observation period numbered 43 (45.3 percent).
Table 1. Prevalence of anti-HCV among hemodialysis patients in 1989

<table>
<thead>
<tr>
<th>Hemodialysis unit</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of patients</td>
<td>No. anti-HCV positive(%)</td>
<td>No. of patients</td>
</tr>
<tr>
<td>A</td>
<td>72</td>
<td>32 (44.4)</td>
<td>60</td>
</tr>
<tr>
<td>B</td>
<td>24</td>
<td>14 (58.3)</td>
<td>18</td>
</tr>
<tr>
<td>C</td>
<td>7</td>
<td>5 (71.4)</td>
<td>13</td>
</tr>
<tr>
<td>D</td>
<td>8</td>
<td>4 (50.0)</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>111</td>
<td>55 (49.5)</td>
<td>93</td>
</tr>
</tbody>
</table>

There were no significant differences in the prevalence of anti-HCV between the sexes and the 4 hemodialysis units.

Table 2. Changes in anti-HCV among hemodialysis patients over a 5 year period (1989-94)

<table>
<thead>
<tr>
<th>Classification by change of anti-HCV</th>
<th>No. of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) + + -&gt; +</td>
<td>105</td>
</tr>
<tr>
<td>2) + + -&gt; -</td>
<td>0</td>
</tr>
<tr>
<td>3) - - -&gt; -</td>
<td>88</td>
</tr>
<tr>
<td>4) - - -&gt; +</td>
<td>11</td>
</tr>
<tr>
<td>Total</td>
<td>204</td>
</tr>
</tbody>
</table>

Table 3. Course of seroconversion of patients (1989 - 94)

<table>
<thead>
<tr>
<th>Case</th>
<th>Unit</th>
<th>Age</th>
<th>Sex</th>
<th>ALT</th>
<th>Ab</th>
<th>RNA</th>
<th>ALT</th>
<th>Ab</th>
<th>RNA</th>
<th>ALT</th>
<th>Ab</th>
<th>RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A</td>
<td>53</td>
<td>M</td>
<td>18</td>
<td>-</td>
<td>-</td>
<td>21</td>
<td>+</td>
<td>+</td>
<td>16</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>A</td>
<td>21</td>
<td>F</td>
<td>8</td>
<td>-</td>
<td>-</td>
<td>59</td>
<td>+</td>
<td>+</td>
<td>54</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>A</td>
<td>56</td>
<td>F</td>
<td>9</td>
<td>-</td>
<td>-</td>
<td>83</td>
<td>+</td>
<td>+</td>
<td>40</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>A</td>
<td>53</td>
<td>M</td>
<td>13</td>
<td>-</td>
<td>-</td>
<td>59</td>
<td>+</td>
<td>+</td>
<td>157</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>A</td>
<td>55</td>
<td>F</td>
<td>15</td>
<td>-</td>
<td>-</td>
<td>16</td>
<td>+</td>
<td>-</td>
<td>9</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>A</td>
<td>28</td>
<td>M</td>
<td>9</td>
<td>-</td>
<td>-</td>
<td>117</td>
<td>-</td>
<td>+</td>
<td>71</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>A</td>
<td>36</td>
<td>M</td>
<td>17</td>
<td>-</td>
<td>-</td>
<td>11</td>
<td>-</td>
<td>-</td>
<td>12</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>A</td>
<td>50</td>
<td>F</td>
<td>6</td>
<td>-</td>
<td>-</td>
<td>8</td>
<td>-</td>
<td>-</td>
<td>9</td>
<td>-</td>
<td>**</td>
</tr>
<tr>
<td>9</td>
<td>C</td>
<td>58</td>
<td>M</td>
<td>40</td>
<td>+</td>
<td>-</td>
<td>31</td>
<td>+</td>
<td>+</td>
<td>21</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>C</td>
<td>41</td>
<td>F</td>
<td>3</td>
<td>-</td>
<td>*</td>
<td>14</td>
<td>+</td>
<td>+</td>
<td>78</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>D</td>
<td>66</td>
<td>M</td>
<td>15</td>
<td>-</td>
<td>-</td>
<td>11</td>
<td>+</td>
<td>-</td>
<td>19</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

ALT: alanine aminotransferase
Ab: antibody to hepatitis C virus
RNA: HCV RNA

*: Transfusion in 1993
**: Transplantation and transfusion in 1990
Table 4. Antibody to hepatitis C virus and hepatitis B surface antigen in 56 hemodialysis patients with liver dysfunction during 5 years observation

<table>
<thead>
<tr>
<th>anti-HCV</th>
<th>HBs Ag</th>
<th>Number of Liver Dysfunction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+</td>
<td>3 *2 (5.3)</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>43 *3 (76.8)</td>
</tr>
<tr>
<td>-</td>
<td>+</td>
<td>1 *4 (1.8)</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>9 (16.1)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>56 (100.0)</td>
</tr>
</tbody>
</table>

anti-HCV; antibody to hepatitis C virus by second-generation assay
HBs Ag; hepatitis B surface antigen
*1: ALT level was elevated at least once during the 5 years
*2: Three patients were positive for HCV RNA and negative for hepatitis B e antigen
*3: 40 of 43 patients were positive for HCV RNA
*4: Positive for hepatitis B e antigen
*5: chi-squared test p<0.001
There were no differences between HBs antigen positive and negative patients

DISCUSSION

This 5-year follow-up revealed anti-HCV seroconversion from negative to positive in 11 of 99 Japanese patients. One patient was HCV RNA positive in the initial test of the serum, therefore, this patient had been infected with HCV prior to start of the investigation. Ten (10.2 percent) of 98 patients (not including one who had been infected before the investigation) were newly infected with HCV during the five year period. The annual rate of HCV infection was 2.0 percent in the 4 hemodialysis units studied.

In three of 11 anti-HCV-seroconverted patients, HCV RNA was detected in the serum in an earlier test. There are at least two possible explanations for this: First, HCV RNA may be more sensitive than anti-HCV, especially in hemodialysis patients 8-10. Second, HCV RNA may be detectable at an earlier stage of infection than is anti-HCV 10. Of these 11 seroconverted patients, we found two HCV RNA-negative patients and one patient who became negative for HCV RNA. We earlier reported that patients with chronic hepatitis C who were treated with interferon remained positive for anti-HCV even after elimination of HCV RNA from their serum 10. Of these reasons, it seems likely that HCV could be eliminated from the serum. Other investigators reported that 60-70 percent of patients with acute hepatitis C developed chronic hepatitis C 13,14. Our data that 80 percent of newly infected patients became HCV carriers is relatively high because immune responses in hemodialysis patients may be inadequate.

Blood transfusion 15,16 and sexual intercourse 17-19 are considerable the routes of HCV infection in our 10 infected patients. It was reported that multiple blood transfusions could be the major source of HCV infection in hemodialysis patients 12,20, but only two of 10 newly infected patients in the present follow-up, had been transfused, except for one given a blood transfusion in 1990. Another patient could not have contracted HCV through blood transfusion in 1993, because donor blood in Red Cross Blood Centers in Japan has been screened by second generation assay since February 1992 and all reactive samples were discarded. Recently there has been little transfusion-related hepatitis linked to HCV infection in Japan 21.

Akahane et al 22 reported that spouses of patients with HCV viremia and chronic liver disease have an increased risk for acquiring HCV, proportional to the duration of marriage. However, that study did not mention other factors that could explain the high prevalence of HCV infection in this age group. From our epidemiological survey of HCV in an endemic area which is located in Fukuoka prefecture, the transmission between spouses can probably be ruled out as the main route of transmission of HCV 22. Moreover, anti-HCV positivity among family members of hemodialysis patients was not higher than in the general population 24. It seems highly unlikely the sexual transmission is the main route of HCV infection in hemodialysis patients.

Allander et al 25 used nucleotide sequencing and found that transmission of HCV was not associated with dialysis machines, rather, it occurred between the patients through unspecified medical treatments in the hemodialysis unit; i.e. injection or venipuncture. Therefore, most of newly infected patients in our study probably contracted HCV during treatments but not through blood transfusion within the hemodialysis units. In hemodialysis units we studied, the staff did not replace the glove on each patient, except for HBs Ag-positive patients. The routes of HCV transmission among hemodialysis patients still have not been found, but the staff working in hemodialysis units must endeavor to avoid transmission. Education in hygienic control for the staff (such as washing hands, using disposable tools, and so on) may be effective in the prevention of HCV transmission.

About half of the hemodialysis patients with HCV viremia had liver dysfunction. The initial prevalence of patients with elevated ALT levels in 1989 was 14.7 percent, the same as that among subjects of the general population 26. However, the prevalence of liver dysfunction in the 5-year observation was three times that of a cross-sectional study, therefore, a large cohort study is needed to evaluate liver dysfunction in patients...
with HCV viremia. Pol et al reported that only 5 of 17 HCV RNA-positive hemodialysis patients had elevations in ALT, even though chronic hepatitis was diagnosed in 16 of these patients by liver biopsy. All patients have not undergone liver biopsy in this study. Therefore, serum ALT levels may not adequately evaluate liver dysfunction for hemodialysis patients, and liver biopsy should probably be done.

The present study confirmed that HCV is an important cause of liver disease in hemodialysis patients and suggests that in hemodialysis patients infected with HCV liver function gradually deteriorates. Because the prognosis and quality of life of patients in end-stage renal failure has greatly improved, it seems likely that many will develop cirrhosis and hepatocellular carcinoma if HCV infection continues. Interferon therapy , which can eliminate the virus from serum, will reduce complications from HCV.

In conclusion, precise hygienic control is needed for hemodialysis patients because HCV infection is occurring in these units through routes other than blood transfusion.

REFERENCES


