Clinical significance of SEN virus infection in patients on maintenance haemodialysis

Nobuhiko Kobayashi¹, Eiji Tanaka¹, Takeji Umemura¹, Akihiro Matsumoto¹, Toshiyo Iijima¹, Makoto Higuchi³, Kazuhiko Hora² and Kendo Kiyosawa¹

¹Second Department of Internal Medicine and ²Division of Artificial Kidney, Shinshu University School of Medicine, Matsumoto, Japan

Abstract

Background. The SEN virus (SENV) has been identified as a putative new hepatitis virus. This study was conducted to clarify the clinical significance of SENV infection in patients on haemodialysis.

Methods. A total of 189 patients on maintenance haemodialysis and 60 healthy controls were enrolled. Of the 189 patients, 154 were followed up for 2 years. SENV DNA (genotypes D and H) was measured by means of polymerase chain reaction.

Results. SENV infection was significantly (P = 0.012) more prevalent in patients on haemodialysis (38%) than in controls (22%). SENV infection was not associated with the amount of transfusion or duration of haemodialysis, while hepatitis C virus (HCV) infection was significantly associated with both of these factors. Elevation of alanine aminotransferase was significantly associated with HCV, but not with SENV viraemia. Of the 154 patients who were followed up, 63 (41%) remained negative, 34 (22%) gained positivity, 28 (18%) lost it and 29 (19%) remained positive for SENV infection. Episodes of alanine aminotransferase elevation were recorded for 3% of the patients who incurred SENV infection and this rate was similar to that observed in patients who were continuously negative for SENV infection (5%).

Conclusions. SENV infection was common in patients on haemodialysis. No evidence was obtained that suggested involvement of the hepatitis virus in the pathogenicity of SENV.

Keywords: haemodialysis; hepatitis; infection; SEN virus

Introduction

Five kinds of hepatitis virus (A–E) have been identified and diagnostic procedures for identification of infections with these viruses have been established. This identification has shown that there are still patients with acute or chronic hepatitis of unknown origin (non-A to E hepatitis) [1,2]. Hepatitis G virus (HGV) [3,4] and TT virus (TTV) [5,6] have been indicated as candidates for new hepatitis viruses. However, most of the findings regarding HGV [7,8] and TTV [9,10] have suggested that those viruses are not causally associated with non-A to E hepatitis.

Recently, a new virus, designated SEN virus (SENV), was identified as a putative non-A to E hepatitis virus (http://ep.espacenet.com/) [11]. This virus contains a single-stranded circular DNA of 3900 nucleotides in length [11] and is distantly related to TTV [12]. SENV can be classified into eight genotypes (A–H) based on the nucleotide sequence [12]. Infections by SENV-D and -H are reportedly involved in transfusion-associated non-A to E hepatitis [13]. However, little is known about their clinical significance.

Patients on haemodialysis are considered to be at risk of infection by blood-borne viruses, such as hepatitis C virus (HCV), because the therapeutic procedures are frequently associated with bleeding and blood transfusions [14,15]. In this study, we tested serum for the presence of SENV-D and -H DNAs in order to clarify the clinical relevance of SENV infection for patients on maintenance haemodialysis.

Subjects and methods

Patients

A list of random numbers was used to randomly select 189 patients (48%) from among 380 Japanese patients who were on maintenance haemodialysis at two hospitals in Nagano Prefecture, Japan, in April 2000. They comprised 119 men
and 70 women, ranging in age from 24 to 83 years. The length of haemodialysis ranged from 30 to 346 months. Past history of blood transfusion was recorded for 93 (49%) patients with a mean transfusion of 3.1 ± 5.8 U. Maintenance haemodialysis had been performed two or three times a week using disposable dialysers with standard bicarbonate dialysates. None of the patients had a history of receiving antiviral therapy, such as interferon. Almost none of the patients with HCV infection were eligible for interferon therapy because of high age, severe complications or normal alanine aminotransferase (ALT) level. Even those who were eligible refused the treatment mainly because of concern about the possible side effects of interferon. Serum samples were collected in April 2000 from the 189 patients who agreed to participate in the present study, 154 of whom had been followed up for at least 2 years and whose serum samples collected in March 1998 were available. Measurements were made for HGV RNA, HGV envelope-2 (E2) antibody and TTV DNA in serum samples collected in 1998 from 45 randomly selected patients.

Sixty apparently healthy Japanese who underwent medical screening for liver diseases in 2000 were enrolled as controls. Mean age (52.6 ± 12.0 years for controls and 61.1 ± 12.2 years for patients; P > 0.2) and percentage of males (50% for controls and 63% for patients; P > 0.2) showed no significant differences between the 60 controls and 189 patients on haemodialysis. None of the controls was positive for hepatitis B surface (HBs) antigen or HCV infection. The HBs antigen and second generation HCV antibody were measured with autoanalysers. The ALT level was measured at least once a month during the follow-up period of 2 years.

**Laboratory tests**

Biochemical liver function tests and peripheral blood cell counts, such as ALT (normal range 11–40 IU/l), white blood cells (WBC; normal range 3500–9800 μl), haemoglobin (normal range 13.4–17.7 g/dl for men and 11.1–15.1 g/dl for women), platelets (normal range 12.7 × 10^11–36.8 × 10^11 μl) were measured with autoanalysers. The ALT level was measured at least once a month during the follow-up period of 2 years.

**Viral markers**

The HBs antigen and second generation HCV antibody were measured with the aid of commercially available enzyme-linked immunosorbent assay (ELISA) kits (International Reagents, Kobe, Japan). Antibody to HGV-E2 protein was detected by using a commercially available ELISA kit (Boehringer Mannheim, Tokyo, Japan).

HCV RNA was detected with the nested polymerase chain reaction (PCR) method using primers in the 5′ non-coding region of the HCV genome, as reported previously [16]. HGV RNA was detected by means of nested PCR with primers in the 5′ non-coding region according to the method reported by Kobayashi et al. [17]. TTV DNA was detected by semi-nested PCR with primers, as reported by Okamoto et al. [6].

The SENV-D and -H DNAs were detected by means of PCR using strain-specific primers as described previously [12]. Briefly, total nucleic acids were extracted from 100 μl of serum with a QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA, USA). The PCR reaction was performed for 40 cycles followed by an extension reaction. PCR products were analysed with a DNA enzyme immunoassay (Diasorin, Saluggia, Italy) with SENV-D and -H specific 5′-end biotinylated probes. Specimens with OD values > 0.350 were considered positive for SENV-D and -H DNAs. The estimated lowest detection level for SENV DNA was 10 copies for each test based on a dilution series of plasmid DNA containing the SENV PCR target insert. This was equivalent to 1600 copies/ml in the originally extracted serum. All positive samples were confirmed by duplicate retesting.

**Statistical analysis**

The unpaired t-test was used to analyse continuous variables and the χ² test was used for analysing categorical data. Where the number of patients was less than five in at least one of the categories, Fisher’s exact test was used. A P-value of < 0.05 was considered significant.

**Results**

SENV (D and/or H) infection was significantly more prevalent (P = 0.012) in the 189 patients on haemodialysis (38%) than in the 60 healthy controls (22%). However, the distribution of SENV-D and -H infections did not significantly differ (P > 0.2) between the patients (SENV-D only 61%, SENV-H only 22%, both SENV-D and -H 17%) and the controls (SENV-D only 77%, SENV-H only 15%, both SENV-D and -H 8%). In the control group, the mean age (SENV positive vs SENV negative: 51.3 ± 8.9 years vs 53.0 ± 12.8 years; P > 0.2) and mean ALT levels (28.4 ± 15.2 IU/l vs 24.1 ± 21.2 IU/l; P > 0.2) did not significantly differ between individuals positive and negative for SENV infection.

Table 1 shows comparisons of clinical and virological backgrounds of patients with and without SENV infection. All backgrounds, except gender, were similar for the two groups of patients. There were 21% more male patients with SENV infection than without it. Among the 71 patients with SENV infection, there was no significant difference in the clinical and virological backgrounds between the patient groups with SENV-D and with SENV-H infection.

The positive rates of HCV antibody and SENV DNA were analysed in relation to the number of transfusion units and the duration of haemodialysis (Figure 1). The rate of HCV antibody positivity was significantly associated with the number of transfusion units (P = 0.004) and the duration of haemodialysis (P = 0.006), while the rate of SENV DNA positivity was not associated with either of them.

Clinical backgrounds were compared among the four groups of patients classified according to changes in SENV infection status (Table 2). The percentage of males was significantly higher in the group of patients whose viraemia continued than in the continuously SENV negative group. Transfusion history and episodes of ALT elevation (maximum 188 IU/l) above the normal upper limit during the follow-up period were similar for the four groups. None of the patients became serum HCV antibody positive during the
follow-up period. Similar ratios of SENV genotypes were seen among the four groups classified according to changes in SENV viraemia: SENV-D only 71%, SENV-H only 12% and both SENV-D and -H 17% in the 34 patients who acquired SENV viraemia, 82, 7 and 11% in the 28 patients who lost SENV viraemia and 38, 17 and 10% in the 29 patients who continued to have SENV viraemia, respectively.

The ALT level was significantly higher in patients with HCV RNA than in those without it, irrespective of age, duration of haemodialysis, and transfusion history. Table 1 provides a comparison of clinical and virological backgrounds of patients with and without SENV infection.

Table 1. Comparison of clinical and virological backgrounds of patients with and without SENV infection

<table>
<thead>
<tr>
<th>Background</th>
<th>SENV (−) (n = 118)</th>
<th>SENV (+) (n = 71)</th>
<th>SENV-D alone (n = 43)</th>
<th>SENV-H alone (n = 16)</th>
<th>Both D and H (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>60.9±11.5</td>
<td>62.7±14.0</td>
<td>58.0±12.6</td>
<td>65.5±12.4</td>
<td>63.2±6.5</td>
</tr>
<tr>
<td>Gender (male %)</td>
<td>55</td>
<td>76</td>
<td>74</td>
<td>75</td>
<td>83</td>
</tr>
<tr>
<td>Duration of haemodialysis (months)</td>
<td>154±93</td>
<td>156±91</td>
<td>153±85</td>
<td>171±81</td>
<td>164±107</td>
</tr>
</tbody>
</table>

Table 2. Comparison of clinical backgrounds among groups classified according to changes in SENV infection during follow-up from 1998 to 2000

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Changes in SENV infection status during 2 year follow-up period</th>
</tr>
</thead>
</table>
| Age (years)  
(years)                    | (−) to (−) (n = 63)  
(−) to (+) (n = 34)  
(+) to (−) (n = 28)  
(+) to (+) (n = 29) |
| Gender (male %)                  | 60.9±11.5          | 60.8±13.3         | 61.2±13.0             | 61.3±12.2             |
| Duration of haemodialysis (months) | 150±95            | 154±88            | 141±91                | 150±96                |
| Transfusion from birth until the year 2000  
(years) | 43% (27)          | 47% (16)          | 46% (13)              | 40% (12)              |
| Transfusion during follow-up  
(years) | 3% (2)            | 3% (1)            | 7% (2)                | 3% (1)                |
| ALT elevation during follow-up  
(years) | 5% (3)            | 3% (1)            | 4% (1)                | 3% (1)                |

aData are expressed as means±SD.
bP<0.001 compared with the continuously SENV negative group. All other comparisons among the four groups were not significant.
cData are expressed as positive % (number).
dPercentage of patients who had an episode of ALT elevation higher than the normal upper limit (number).

follow-up period. Similar ratios of SENV genotypes were seen among the four groups classified according to changes in SENV viraemia: SENV-D only 71%, SENV-H only 12% and both SENV-D and -H 17% in the 34 patients who acquired SENV viraemia, 82, 7 and 11% in the 28 patients who lost SENV viraemia and 38, 17 and 10% in the 29 patients who continued to have SENV viraemia, respectively.

The ALT level was significantly higher in patients with HCV RNA than in those without it, irrespective
of the status of SENV DNA. In fact, the ALT levels (means ± SD) according to the status of HCV and SENV infections were 14 ± 11 IU/l in the 15 patients who were both negative for HCV RNA and SENV DNA (group 1), 14 ± 10 IU/l in the 10 patients who were negative for HCV RNA but positive for SENV DNA (group 2), 27 ± 17 IU/l in the 10 patients who were positive for HCV RNA but negative for SENV DNA (group 3) and 28 ± 16 IU/l in the six patients who were both positive for HCV RNA and SENV DNA (group 4) (group 1 vs group 3, P = 0.032; group 2 vs group 3, P = 0.011; group 1 vs group 4, P = 0.009; group 2 vs group 4, P = 0.013). The WBC and platelet counts and haemoglobin concentrations in peripheral blood did not differ significantly among the four groups.

A comparison of the positive rates of HGV RNA, HGV-E2 antibody and TTV DNA between SENV infection positive and negative patients is shown in Table 3. Rates of HGV and TTV infections were similar for the patients with and without SENV infection.

### Discussion

The prevalence of SENV infection reported by Umemura et al. [13] was 1.8% for blood donors in the USA, but 10% among Japanese blood donors according to the findings of Shibata et al. [18]. Our study showed that as many as 22% of healthy individuals in Japan were infected with SENV. These results suggest that considerably more people in Japan (similar to findings in Taiwan [19]) are infected with SENV than in the USA. Although SENV infection is, thus, certainly not low among Japanese in general, the prevalence in patients on maintenance haemodialysis (38%) was significantly higher, prompting us to analyse the clinical significance of SENV infection in those patients. Our analysis combined SENV-D and -H infections as SENV infection because we found no significant difference in clinical or virological backgrounds between the two SENV genotypes.

The findings of Umemura et al. [13] strongly suggest that SENV is transmitted through blood transfusion. However, our results showed that SENV was not associated with blood transfusion history or duration of haemodialysis while infection with HCV, which is a well-known blood-borne hepatitis virus, was significantly associated with both factors. One possible explanation for this discrepancy is that SENV infection is highly prevalent in the Japanese population, another that SENV can be transmitted through not only parenteral but also feco-oral routes. This possibility is supported by the finding that TTV, which is distantly related to SENV, can be transmitted via either route [6]. Transmission of SENV through haemodialysis materials was considered to be negligible because all the materials were disposable.

Loss of SENV infection was observed in about half of the patients during the 2-year follow-up. This high incidence of loss of SENV viraemia seemed to occur naturally because none of the patients was treated with anti-viral agents such as interferon. Umemura et al. [20] reported that SENV viraemia disappeared in 55% of their subjects after 6 months and in 74% 5 years after a new SENV infection. These results suggest that natural cessation of the SENV carrier state is not a rare event. As many as 35% of the patients who were previously negative for SENV infection acquired it during the follow-up period. No notable differences in backgrounds, including history of blood transfusion, were observed among the patients who gained SENV viraemia. These findings indicate that new SENV infection is a common occurrence among patients on haemodialysis and is transmitted mainly through an as yet unidentified transmission route.

Serum levels of ALT were associated with HCV, but not with SENV viraemia in the cross-sectional analysis. Episodes of ALT elevation during the 2-year follow-up were recorded in only 3% of the patients who acquired SENV viraemia even though serum ALT levels were measured monthly. Furthermore, the number of episodes was similar for patients who acquired SENV viraemia and those who did not. These results suggest that SENV infection is not associated with the occurrence of hepatitis.

Since data on HGV and TTV infections were available from 45 serum samples collected in 1998, we analysed the association between these and SENV infections. The results suggest that SENV infects humans independent of the occurrence of HGV and TTV.

Male patients tended to retain SENV viraemia longer than female patients. This tendency may account for the higher prevalence of SENV infection in male patients on haemodialysis. No gender difference for SENV infection has been reported in healthy individuals or in patients with liver diseases [13,21], but ours is the first study on SENV infection in patients on haemodialysis. Thus, further investigations

### Table 3. Comparison of HGV- and TTV-related markers for patients with and without SENV infection

<table>
<thead>
<tr>
<th>Viral marker</th>
<th>SENV DNA (+) (n = 15)</th>
<th>SENV DNA (-) (n = 30)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>HGV RNA</td>
<td>7% (1)</td>
<td>10% (3)</td>
<td>NS</td>
</tr>
<tr>
<td>HGV-E2 antibody</td>
<td>7% (1)</td>
<td>7% (2)</td>
<td>NS</td>
</tr>
<tr>
<td>TTV DNA</td>
<td>40% (6)</td>
<td>43% (13)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data are expressed as positive % (number). NS, not significant.
are necessary to determine whether this male preponderance of SENV infection is, indeed, characteristic of patients on maintenance haemodialysis.

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