

## Original Article

# SEN Virus Prevalence among Non-B and Non-C Hepatitis Patients with High Liver Function Tests in the South of Turkey

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**SUMMARY:** We investigated the characteristics and detection rates of SEN virus (SENV) infection among 100 Turkish patients who had with high alanine aminotransferase (ALT) and aspartate aminotransferase levels but were negative for HBV DNA and HCV RNA and had no history of transfusion. As a control group, we also analyzed 50 healthy individuals who had normal ALT levels, were negative for HBV DNA and HCV RNA, and had no history of transfusion. The serum samples of patient and controls were analyzed by PCR to detect the presence of SENV DNA and its two genotypes (SENV-H and SENV-D). We detected SENV DNA in 13 of 100 (13%) patients. Five of 13 (38.46%) patients were positive for SENV-D and 8 of 13 (61.53%) patients were positive for SENV-H DNA. We also detected SENV DNA in 5 of 50 (10%) patients in the control group. Two of 5 (40%) patients were positive for SENV-D and 3 of 5 (60%) patients were positive for SENV-H DNA in the control group. SENV was detected at almost the same frequency in the patient and control groups. SENV did not seem to contribute to the pathogenesis of liver disease ( $P > 0.05$ ) in this cohort. Our results also showed that SENV transmission was not only associated with blood transfusion but also with some other possible routes.

## INTRODUCTION

Five hepatitis viruses (A-E) cause more than 80% of cases of viral hepatitis. However, the fact that nearly 20% of individuals with acute hepatitis test negative for all known hepatitis viruses, as do up to 10% of patients with transfusion-associated hepatitis, suggests the existence of other viral hepatitis agents (1).

SEN virus (SENV) is a single-stranded, circular DNA virus of approximately 3,900 nucleotides that lacks an envelope (1,2). The SENV genome contains at least three open reading frames (2). Phylogenetic analysis of SENV has demonstrated the existence of eight strains or genotypes, which have been designed strains A-H (3). The characteristics of SENV that have been determined to date show a remarkable parallel with those of the TT virus (TTV), and it may be that the viruses share a common ancestor (2). Like TTV, SENV has been classified into the *Circoviridae* family (4).

Active infection is frequent in healthy blood donors and in the general population. However, this high prevalence can be attributed to only some of the SENV strains; for example, SENV-B, SENV-A and SENV-E are less frequently found among blood donors and do not appear to be related to non-A-E hepatitis (3,4). In contrast, genotypes D and H have been found in only 1% of blood but in more than 50% of non-A-E hepatitis cases. Chronic infection is detected in patients with various hepatic diseases (4). Despite the favorable ratio of donors to patients with acute hepatitis for SENV-D and -H

and the fact that preliminary data suggest that SENV can replicate in the liver, no true association between SENV and liver damage has been proven so far (5). Little is known of the natural history of the infection. Chronic infections of over 10-year duration have been observed in retrospectively tested samples of infected individuals, but most patients clear viremia during the first months of exposure. Therefore, true exposure to virus is difficult to assess, as no serological test for SENV antibodies is currently available (4).

SENV is transmitted by blood, as demonstrated by comparing the sequence homology between donors and recipients (6). Moreover, transfused patients are at higher risk of acquiring SENV than are nontransfused patients. Risk of infection in transfused patients increased proportionally with the number of units of blood transfused (7). However, many studies suggest that there is no association between SENV and liver pathology. To clarify the characteristics and prevalence of SENV infection among our patients, we investigated the detection rates of serum SENV DNA in the patient and control groups.

## MATERIALS AND METHODS

**Serum samples and study populations:** Serum samples were obtained from patients who were enrolled at the Cukurova University Department of Gastroenterology between July 2003 and March 2004. All serum samples were collected at  $-24^{\circ}\text{C}$ . Subjects comprised 54 males and 46 females with a mean age of  $41.31 \pm 9.33$ . Patients who were negative for hepatitis B virus (HBV) DNA and hepatitis C virus (HCV) RNA, or who were frequently below the upper limit of normal (ULN) for alanine aminotransferase (ALT) or aspartate aminotransferase (AST), were not included in the study (the ULN for ALT was 47 IU/L and that for AST

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was 46 IU/L). No patient had a transfusion history. Fifty serum samples were also obtained from healthy individuals, i.e., 25 males and 25 females with a mean age of  $39.56 \pm 7.37$  who showed normal levels of ALT and AST, were negative for HBV DNA and HCV RNA, and had no history of transfusion. The control serum samples were also obtained and collected during the same period and under the same conditions as described above. The clinical data are summarized in Table 3.

**Biochemical liver function tests:** Liver biochemical tests, including ALT (normal range, 7-46 IU/L), AST (8-46 IU/L) and total bilirubin (0.1 - 1.5 mg/dL) levels, were done for all patients at the initial examination.

**PCR amplifications for HBV and HCV:** HCV RNA was assayed with the nested polymerase chain reaction (PCR) method using primers in the 5' non-coding region of the HCV genome, as reported previously (8). HBV DNA was also assayed with PCR amplification using primers in the polymerase gene of the HBV genome, as reported previously (9). Subjects showing positivity by either PCR were not included in the study.

**Extraction of SENV DNA:** Serum (100  $\mu$ L) was mixed with 300  $\mu$ L of lysis buffer (13.3 mmol/L Tris-HCl [pH 8.0], 6.7 mmol/L ethylenediaminetetraacetic acid, 0.67% sodium dodecyl sulphate, 133  $\mu$ g/mL proteinase-K) and incubated at 56°C for 4 h. Two phenol-chloroform extractions were followed by one chloroform extraction, and DNA was precipitated with ethanol. The precipitate was dissolved in 25  $\mu$ L of TE buffer (10 mmol/L Tris-HCl [pH 8.0], 1 mmol/L ethylenediaminetetraacetic acid).

**Detection of SENV DNA:** SENV DNA was determined by PCR using the primers reported by Umemura et al. (5) and Kojima et al. (10). For general SENV screening, a primer pair was used to the conserved region among all SENV genotypes A-I with sense primer AI-1F and antisense primer AI-1R (Table 1). The PCR mixture consisted of 0.5  $\mu$ M of the forward primer and 0.5  $\mu$ M of the reverse primer in a 50  $\mu$ L mixture containing PCR buffer, 0.25 mmol/L dNTPs, 2.5 mmol/L magnesium chloride, 1.25 U Taq polymerase (Promega, Madison, Wis., USA) and 5  $\mu$ L of extracted DNA. PCR amplification consisted of a single cycle of 60 sec at 95°C for denaturation, followed by 35 cycles of amplification at 95°C for 30 sec, 68°C for 60 sec.

For SENV-D detection, the primers D10S and L2AS (Table 1) were used in a 50  $\mu$ L PCR mixture containing PCR buffer,

0.25 mmol/L dNTPs, 2.5 mmol/L magnesium chloride, 1.25 U Taq polymerase (Promega) and 5  $\mu$ L of extracted DNA. The PCR was performed in a thermal cycler (Eppendorf Mastercycler, Hamburg, Germany) using the following protocol: 40 cycles of 94°C for 1 min, 58°C for 1 min, and 72°C for 1.5 min, followed by an extension reaction at 72°C for 10 min. SENV-H detection was performing using the primers C5S and L2AS (Table 1) and the same PCR conditions as described above. The PCR products were separated by 2% agarose gel and stained by ethidium bromide.

**Statistical analysis:** Differences between groups were examined by  $\chi^2$  and Fisher's exact test. A *P* value of <0.05 was considered to be significant. Data in the tables are expressed as the means  $\pm$  standard deviation (SD).

## RESULTS

SENV DNA was detected in 13 (13%) of the 100 patients and 5 (10%) of the 50 control subjects by screening PCR with AI-1F and AI-1R general primers. The prevalences of SENV DNA in the patient and control groups are shown in Table 2. No significant differences were found in the prevalence of SENV-positive cases between the patient and control groups. Although the SENV-positive cases had relatively high ALT, AST and total bilirubin levels in both the patient and control groups, no significant association (*P* > 0.05) was found between SENV and liver disease (Table 3).

By genotype-specific PCR with genotype D-specific primers, SENV-D was detected in 5 of 13 SENV-positive cases (38.46%) in the patient group. With genotype H-specific primers, SENV-H was detected in 8 of 13 SENV-positive cases (61.53%) in the patient group. In the control group, SENV-D was detected in 2 (40%) of 5 subjects and SENV-H was detected in 3 (60%) of 5 SENV-positive cases. There was also no significant difference in either SENV-D or SENV-H cases between the patient and control groups. Although most SENV-positive cases were male, no statistically significant differences in sex were found between SENV-positive and SENV-negative cases (*P* > 0.05). Similarly, no significant differences in age were found between SENV-positive and SENV-negative cases. All data are summarized in Table 3.

Table 1. Primers used to detect SENV\*

Sense primer (Sequence)	Antisense primer (Sequence)	Region amplified (nt.)
AI-1F (5'-TWCYCMAACGACCAGCTAGACCT-3')	AI-1R (5'-GTTTGTGGTGAGCAGAACGGA-3')	1028-1377 <sup>1)</sup> 987-1336 <sup>2)</sup>
D10S (5'-GTAACCTTGC GG TCAACTGCC-3')	L2AS (5'-CCTCGGTTKSA AAKGTYTGATAGT-3')	1322-1544 <sup>1)</sup>
C5S (5'-GGTGCCCTWGTYAGTTGGCGGT-3')	L2AS (5'-CCTCGGTTKSA AAKGTYTGATAGT-3')	1271-1500 <sup>2)</sup>

W, A or T; Y, C or T; M, A or C; K, G or T; S, C or G.

<sup>1)</sup>: Obtained from the sequence of SENV-D (AX025730).

<sup>2)</sup>: Obtained from the sequence of SENV-H (AX025838).

\*These data were taken from the study by Kojima et al. (10)

Table 2. Prevalence of SENV infections in patient and control groups

Study group	Total	SENV positive no. (%)	SENV-D positive no. (%)	SENV-H positive no. (%)
Patient group	100	13 (13)	5 (38.46)	8 (61.53)
Control group	50	5 (10)	2 (40.00)	3 (60.00)

Table 3. Clinical features of patient and control groups

Characteristics	Patient group (n = 100)				Control group (n = 50)			
	SENV Positive (n = 13)	SENV Negative (n = 87)	SENV-D Positive (n = 5)	SENV-H Positive (n = 8)	SENV Positive (n = 5)	SENV Negative (n = 45)	SENV-D Positive (n = 2)	SENV-H Positive (n = 3)
Age <sup>1)</sup>	45 ± 10	40 ± 9	44 ± 6	46 ± 12	43 ± 9	39 ± 7	46 ± 15	41 ± 5
Sex (F/M)	4/9	42/45	2/3	2/6	-/5	25/20	-/2	-/3
ALT (IU/L) <sup>1)</sup>	100 ± 23	74 ± 15	114 ± 32	91 ± 8	38 ± 4	25 ± 10	35 ± 2	41 ± 3
AST (IU/L) <sup>1)</sup>	91 ± 20	69 ± 13	102 ± 29	84 ± 8	35 ± 4	19 ± 10	30 ± 4	37 ± 2
TB (mg/dL) <sup>1)</sup>	2.8 ± 1.4	2.2 ± 1.6	2.6 ± 1.5	2.0 ± 1.4	0.9 ± 0.5	1.2 ± 0.7	1.0 ± 0.8	1.1 ± 0.8

<sup>1)</sup>: Data are expressed as the means ± standard deviations.

F, female; M, male; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TB, total bilirubin.

## DISCUSSION

Whether SENV causes liver disease remains unclear. Supportive data include the appearance of SENV DNA in the blood of blood transfusion recipients who develop post-transfusion hepatitis and the high prevalence rates of SENV infection among patients with chronic liver disease and/or risk factors for chronic liver disease (3). On the other hand, high SENV prevalence rates also exist in the general population, the majority of whom are not thought to have liver disease (11,12). The prevalence of SENV infection has been reported as 1.8% for blood donors in the USA by Umemura et al. (5), but as 10% among Japanese blood donors by Shibata et al. (13). In addition, Kobayashi et al. reported that SENV infection was significantly more prevalent in their patients on hemodialysis (38%) than in controls (22%) (14).

For the present study, we used the primers reported by Umemura et al. (5) and Kojima et al. (10). Kojima et al. (10) have reported that the sensitivity and specificity of the genotype-specific primers were 100% and 100% for SENV-D, respectively. For SENV-H, however, while the sensitivity was 100%, the specificity was 64%. Therefore, our results for genotype H might require confirmation by hybridization or sequencing. However, they suggest that screening of PCR products amplified with general primers (AI-1F and AI-1R) could serve as a useful SENV-screening assay.

The findings of Umemura et al. (5) and Kobayashi et al. (14) strongly suggest that SENV is transmitted through blood transfusion. However, our results showed that SENV was not associated not only with blood transfusion but also some other possible routes such as fecal-oral transmission. The finding that TTV, which is distantly related to SENV, can be transmitted via either route (15) supports this possibility. This is also supported by the fact that the SENV prevalence in patients with acute hepatitis A infection, which is transmitted by the fecal-oral route, is higher than in healthy adults (16). Pirovano et al. (17) studied mother to infant transmission of SENV infection. In their study SENV was identified in 15 of the 30 women, and 13 of the 30 babies became positive. These data suggest that vertical transmission of SENV does occur. All these data may explain the high prevalence rates of SENV in the normal population.

Yoshida et al. (18) have reported that there were no significant differences in age, sex, liver function, history of blood transfusion, or amount of alcohol intake between SENV-positive and SENV-negative chronic liver disease and hepatocellular carcinoma (HCC) patients. They have also reported that SENV was detected at almost the same frequency in patients with and without liver disease, and that SENV did not seem to contribute to the pathogenesis of liver disease

(18). Our results are in agreement with these data.

According to the findings of Umemura et al. (5), Kobayashi et al. (14), Pirovano et al. (17), and Yoshida et al. (18), there is no association between SENV and liver pathology. Our findings are in agreement with these findings. Therefore, although it remains unclear whether SENV causes liver disease, further studies should be performed to explain the presence of SENV in patients without any liver pathology.

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