Original Article

SEN virus infection in Egyptian patients undergoing maintenance hemodialysis: prevalence and clinical importance

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Background and purpose: SEN virus (SENV) is assumed to be responsible for post-transfusion non-A to -E hepatitis. Phylogenetic analysis of SENV has shown 9 different strains. Two strains, SENV-H and SENV-D, were described as possible candidates for post-transfusion hepatitis. This study examined the prevalence of SENV infection and its clinical importance for patients undergoing hemodialysis.

Methods: Serum samples were obtained from 63 long-term hemodialysis patients, and examined for SENV-H and SENV-D viremia by polymerase chain reaction. Serum samples were also obtained from 20 patients with chronic kidney diseases (CKD) who were not undergoing hemodialysis and from 20 apparently healthy blood donors to act as controls. For SENV screening, a primer pair was used for the conserved ORF1 region among all SENV genotypes from A to I.

Results: SENV infection was significantly more frequent among hemodialysis patients (33/63; 52.4%) and those with CKD (10/20; 50.0%) than among the control participants (2/20; 10.0%) [$p = 0.003$]. Twenty three of 33 hemodialysis patients had SENV-H or -D, 61% of whom were positive for SENV-H only, 4% were positive for SENV-D only, and 36% were positive for both SENV-H and SENV-D. SENV infection was not associated with age, sex, amount or duration of hemodialysis, or liver function test results. Elevated alanine aminotransferase was significantly associated with HCV viremia, but not with SENV infection.

Conclusions: Egyptian hemodialysis patients and those with CKD are at higher risk for SENV transmission. SENV-H is more prevalent than SENV-D.

Key words: Polymerase chain reaction; Prevalence; Renal dialysis; Torque teno virus

Introduction

Recently, a new virus, designated SEN virus (SENV), was isolated from serum of an HIV-positive patient who was an injection drug abuser. SENV is thought to be transmitted parenterally and to cause post-transfusion hepatitis in humans [1]. The genome of SENV is similar to that of the torque teno virus (TTV), and both are classified within the circovirus family. Both SENV and TTV are single-stranded non-enveloped DNA viruses of 3800 nucleotides [2]. Although structurally similar,

the genomes of SENV and TTV have less than 55% nucleotide sequence homology and less than 37% amino acid homology. Of 9 SENV strains that have been identified by phylogenetic analysis, 2 (SENV strain H [SENV-H] and SENV strain D [SENV-D]) have been postulated to be candidate viruses for post-transfusion hepatitis [2].

Patients undergoing hemodialysis are known to be at increased risk for acquiring blood borne transmitted infections such as hepatitis C virus (HCV) [3]. It has been documented that the routes of SENV infection might be mostly parenteral, for example, via blood transfusion, intravenous drug use, or hemodialysis [4,5]. Moreover, SENV infection is frequently observed in patients with hepatitis B virus (HBV; 23%

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to 59%) and HCV (22% to 89%), and patients with hepatitis of unknown etiology [6].

TTV prevalence rates range from 2% to 53% [7], and SENV prevalence rates range from 13% to 68% in hemodialysis patients [2]. Although SENV has been observed in patients with acute and chronic liver disease of unknown etiology, the role of SENV in the pathogenesis of liver disease is not yet known [8].

The aim of this study was to establish a molecular biological method to ascertain the prevalence of SENV infection in hemodialysis patients, evaluate the clinical importance of SENV infection in hemodialysis patients, and study the effect of certain risk factors (duration of hemodialysis, number of blood transfusions, number of surgeries, and coinfection with HCV or TTV) on viral transmission.

Methods

Patients

Serum samples from 103 participants were studied from July 2007 to Jan 2008. The study participants were divided into 3 groups, as follows: group 1 included 63 random long-term hemodialysis patients at the Ahmed Maher Teaching Hospital (AMTH), Cairo, Egypt; group 2 comprised 20 patients with chronic kidney disease (CKD) who had not yet started hemodialysis at the AMTH; and group 3 included 20 apparently healthy participants who had donated blood at the National Cancer Institute, Cairo University, Cairo. Groups 2 and 3 acted as control patients. Informed consent was obtained from all study participants.

In group 1, there were 27 men and 36 women. The mean ± standard deviation (SD) age was 48.68 ± 15.90 years. The median duration of hemodialysis was 52 months (range, 12-156 months). Past history of blood transfusion was recorded for 42 patients (66.7%), with a mean ± SD transfusion rate of 2.27 ± 2.42 pints. Maintenance hemodialysis was performed 3 times a week using disposable dialysers with standard bicarbonate dialysates. None of the patients had a history of receiving antiviral therapy and none were positive for HBV. Group 2 included 11 men and 9 women. The mean ± SD age was 44.45 ± 9.50 years. Group 3 comprised 16 men and 4 women. The mean age 28.95 ± 8.75 years. None of the control participants was positive for HBV DNA or HCV RNA, and they had normal alanine aminotransferase (ALT) levels, with no history of blood transfusion.

Patients with a history of antiviral therapy and patients known to be HBV positive were excluded.

Laboratory tests

All serum samples were frozen at –20°C until analysis. Biochemical liver function tests and peripheral blood cell counts, such as ALT (normal range, 11-40 IU/L), white blood cells (normal range, 3500-9800/μL), hemoglobin (normal range, 13.4-17.7 g/dL for men and 11.1-15.1 g/dL for women), and platelets (normal range, $12.7\text{-}36.8 \times 10^4/\mu L$) were measured.

Viral markers

The hepatitis B surface antigen and HCV antibody were measured by commercially available enzyme-linked immunosorbent assay kits. Each serum sample was extracted using a Vitek extraction kit for purification of both RNA and DNA nucleic acid. HCV RNA was detected by polymerase chain reaction (PCR), as described by Zekri et al [9]. Ten microliters of the amplicons were analyzed by electrophoresis through a 1.2% ethidium bromide–stained agarose gel and DNA was transferred from the gel onto a nitrocellulose filter with 4 N sodium hydroxide. The transferred DNA was cross-linked by incubation for 2 to 3 h at 80°C and the blot was then hybridized with an internal probe.

TTV-DNA was detected by semi-nested PCR using primers NG059, NG061, and NG063. During the first amplification, the primers targeted the nucleotide positions from 1920 to 2205. In the second amplification (semi-nested) the primers targeted the nucleotides from 1935 to 2205 to give amplification products of 270 bp, and then were visualized on ethidium bromide–stained 2% agarose gel [10].

SENV-DNA was detected using a primer pair targeted to the conserved ORF1 region of all SENV genotypes from A to I between nucleotides 987 and 1377. A second round of PCR was performed for detection of the SENV-D or SENV-H genotype. For SENV-D detection, the primers used amplified D gene between nucleotides 1148 and 1341. For SENV-H detection, the primers used amplified H gene between nucleotides 1080 and 1198. The amplification products (384-bp fragments for SENV, 193-bp fragments for SENV-D, and 118-bp fragments for SENV-H) were separated by electrophoresis on 2% agarose gel, stained with ethidium bromide, and visualized using an ultraviolet transilluminator [11]. In each PCR assay, 1 negative and 2 positive samples were tested together with the serum samples. Reliability of the PCR methods to detect SENV DNA was done for 5 randomly selected samples from each group of SENV-D

DNA (+), SENV-D DNA (–), SENV-H DNA (+), and SENV-H DNA (–) for repeat testing.

Statistical analysis

Data were analyzed using the Statistical Package for the Social Sciences for Windows (Version 15; SPSS, Inc., Chicago, IL, USA). Numerical data were expressed as mean ± SD, median, and range. Qualitative data were expressed as frequency and percent. Chi-squared test (Fisher's exact test) was used to examine the relationship between qualitative variables. For quantitative data, comparison between 2 groups was done using Mann-Whitney U test. Comparison between 3 groups was done using Kruskal-Wallis test. A p value of ≤0.05 was considered significant.

Results

PCR screening using general primers showed that SENV infection was significantly more prevalent among hemodialysis patients (33/63; 52.4%) and those with CKD (10/20; 50.0%) than among the 20 apparently healthy blood donors (2/20; 10.0%) [$p = 0.003$; Fig. 1]. Genotype-specific PCR using primers for SENV-H and -D genotypes showed that 23 of the 33 hemodialysis patients with SENV (70.0%) were positive for SENV-H and/or -D (Fig. 2). The remaining 10 patients (30%) had non-H non-D SENV. SENV-H alone was detected in 14 of 23 SENV-positive patients (61.0%), while SENV-D alone was detected in 1 patient (4%); both strains were detected in 8 patients

(36%). In the control group, only 2 individuals were positive for SENV, 1 of whom had SENV-D only and 1 had SENV-H only.

The frequency of TTV DNA infection was not significantly different between the hemodialysis patients and the control group (36.5% vs 30.0%, respectively; $p = 0.193$). Table 1 shows the prevalence of viral infections among the 3 groups.

In the hemodialysis group, ALT levels did not differ significantly between SENV-positive and -negative patients (33.0 ± 8.7 vs 31.9 ± 8.9, respectively; $p = 0.725$). The ALT levels also did not differ between TTV-positive and -negative patients in this group (32.0 ± 8.3 vs 32.8 ± 9.1, respectively; $p = 0.593$). Table 2 shows the clinical features of the hemodialysis patients in relation to the viral infections.

Positive TTV-DNA was significantly associated with a higher number of blood transfusion units ($p = 0.007$). SENV-DNA positivity and HCV positivity was not associated with number of transfusion units or duration of dialysis.

TTV infection was more frequent among hemodialysis patients with positive SENV-DNA (45%) than among those with negative SENV-DNA (26%), but this was not statistically significant (Table 3). There was no significant difference in the frequency of SENV infection between patients with active HCV infection (HCV antibody–positive and HCV RNA–positive) and those with a history of HCV infection (HCV antibody–positive and HCV RNA–negative) [5/10 (50%) vs 11/18 (61%), respectively; $p = 0.778$].
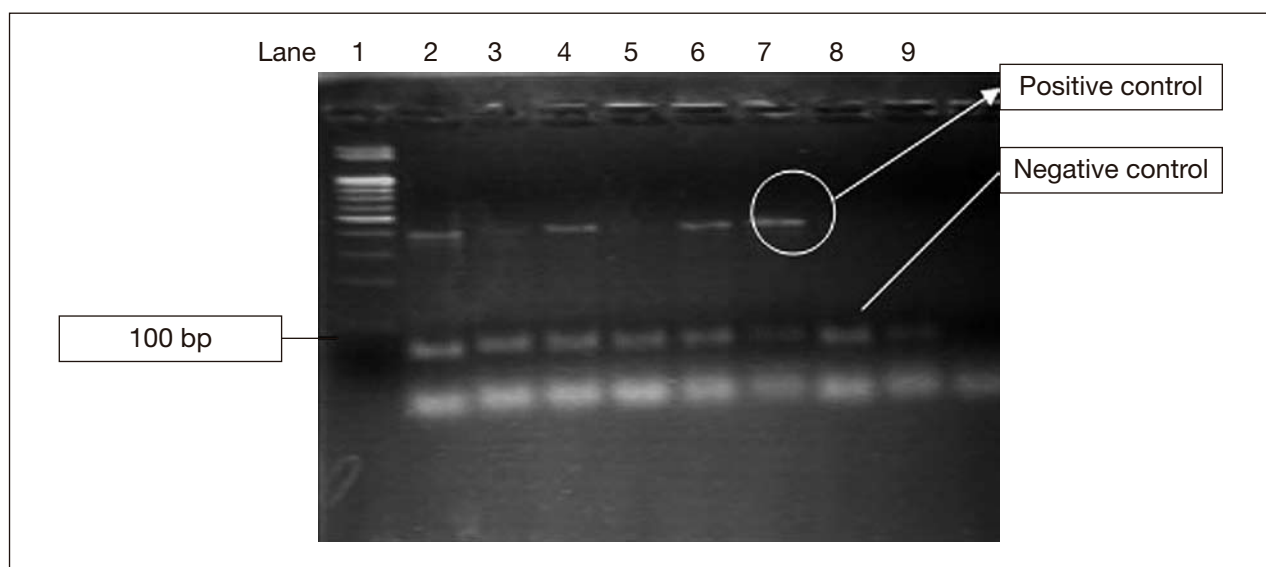

Fig. 1. Ethidium bromide–stained gel electrophoresis of SEN virus DNA polymerase chain reaction product showing positive and negative lanes. Positive signals are 384 bp. Lane 1 = 100-bp ladder; lanes 2-7 = positive lanes; lanes 8, 9 = negative lanes.
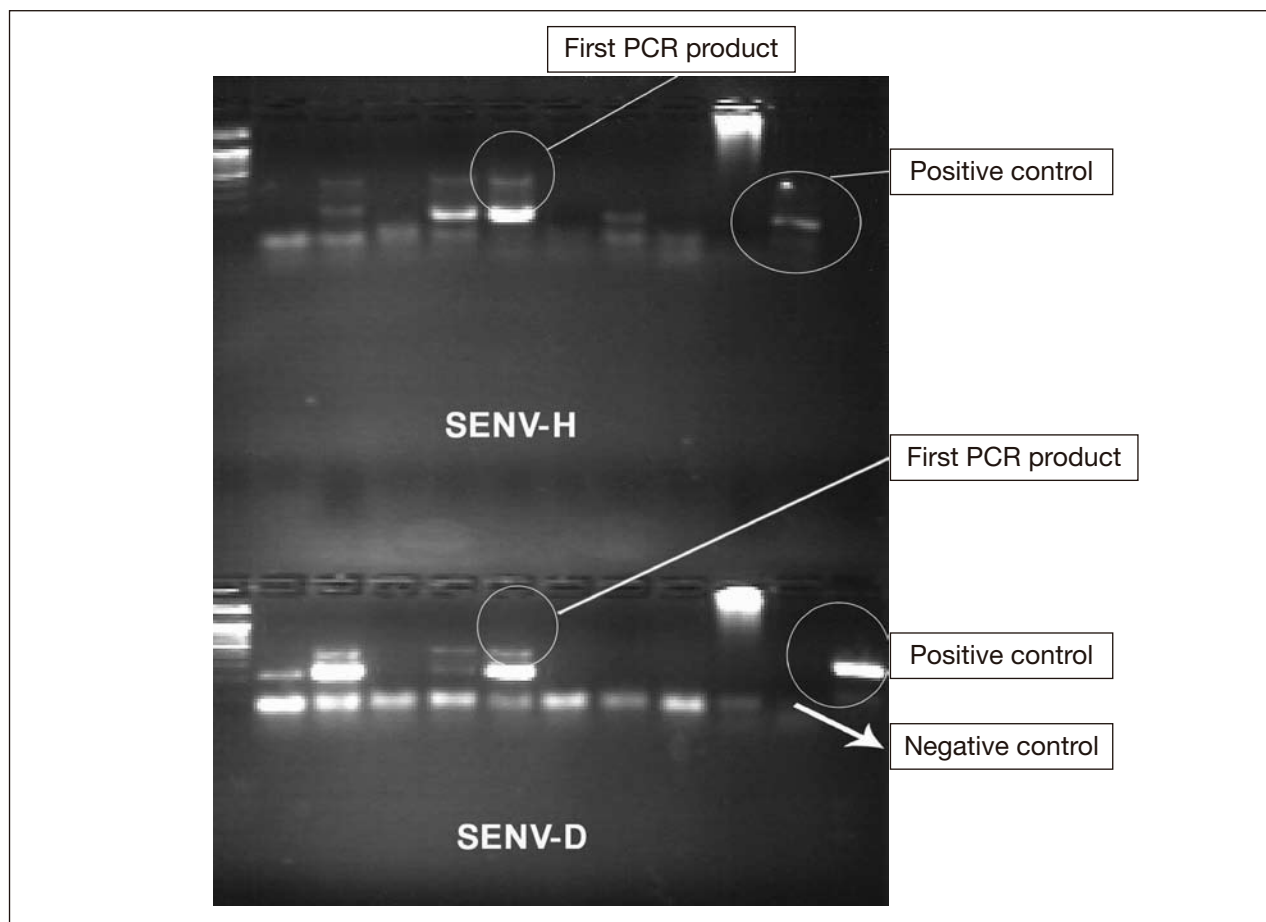
Fig. 2. Ethidium bromide–stained gel electrophoresis of SEN virus type H and SEN virus type D polymerase chain reaction (PCR) products showing SEN virus type H–positive lanes at 118-bp, SEN virus type D–positive lanes at 193 bp, and respective negative lanes. Lane 1 = 100-bp ladder; lanes 3, 5, 6, 8, 11 = SEN virus type H–positive lanes; lanes 2, 4, 7, 9, 10 = SEN virus type H–negative lanes; lanes 2, 3, 5, 6, 12 = SEN virus type D–positive lanes; lanes 7, 8, 9, 10, 11 = SEN virus type H–negative lanes.

Table 1. Prevalence of SEN virus (SENV), hepatitis C virus (HCV), and torque teno virus (TTV) infections among patients undergoing hemodialysis, patients with chronic kidney disease, and healthy controls.

Viral marker	Hemodialysis (n = 63) No. (%)	Chronic kidney disease (n = 20) No. (%)	Controls (n = 20) No. (%)	p
SENV DNA positive	33 (52.4)	10 (50.0)	2 (10.0)	0.003
SENV-H positive	23 (36.5)	10 (50.0)	1 (5.0)	0.007
SENV-D positive	9 (14.3)	1 (5.0)	1 (5.0)	0.478
SENV DNA positive; non-H non-D	10 (16.0)	0 (0)	0 (0)	0.037
TTV DNA positive	23 (36.5)	3 (15.0)	6 (30.0)	0.193
HCV antibody positive	28 (44.4)	5 (25.0)	0 (0)	0.191
HCV RNA positive	14 (22.0)	5 (25.0)	0 (0)	0.190

ALT levels were significantly lower in HCV antibody–positive patients infected with SENV than in patients with HCV antibody without SENV (33.1 ± 6.0 vs 49.3 ± 1.2, respectively; $p = 0.03$).

Ten of 63 hemodialysis patients (16%) had undergone at least 1 surgical procedure, 6 of whom (60%) were positive for SENV infection.

Discussion

SENV, a novel DNA virus, has been found to be associated with post-transfusion non-A to -E hepatitis [8]. Currently, no data are available for SENV infection among hemodialysis patients in Egypt. For this study, a nested version of PCR capable of detecting and

Table 2. Clinical features of hemodialysis patients in relation to the type of virus detected.

Characteristic	SEN virus			Hepatitis C virus antibody			Torque teno virus		
	Positive (n = 33)	Negative (n = 30)	p	Positive (n = 28)	Negative (n = 35)	p	Positive (n = 23)	Negative (n = 40)	p
Age (years; mean ± SD)	49.2 ± 14.1	48.2 ± 17.9	0.912	50.1 ± 14.7	47.5 ± 16.9	0.647	51.5 ± 12.3	47.1 ± 17.6	0.327
Sex (male:female)	17/16	10/20	0.145	11/17	16/19	0.608	8/15	19/21	0.326
Duration of dialysis (years; median) [range]	4.0 (1-13)	4.0 (1-11)	0.649	4.5 (2-11)	4.0 (1-13)	0.202	5.0 (1-13)	4.0 (1-11)	0.356
No. of blood units transfused (median [range])	2.0 (0-11)	2.0 (0-6)	0.315	2.0 (0-11)	2.0 (0-6)	0.351	3.0 (0-8)	1.0 (0-11)	0.007
Alanine aminotransferase (IU/L; mean ± SD)	33.0 ± 8.7	31.9 ± 8.9	0.440	34.9 ± 10.7	30.5 ± 6.3	0.119	32.0 ± 8.3	32.8 ± 9.1	0.819
Multiple surgical procedures No. (%)	6.0 (18.0)	4.0 (13.0)	0.735	3.0 (10.7)	7.0 (20.0)	0.490	3.0 (13.0)	7.0 (17.5)	0.734

Abbreviation: SD = standard deviation.

Table 3. Prevalence of infection among 63 hemodialysis patients with more than 1 viral marker.

Viral marker	SENV positive (n = 33) No. (%)	SENV negative (n = 30) No. (%)	p
TTV DNA positive (n = 23)	15 (45)	8 (26)	0.122
HCV antibody positive (n = 28)	15 (45)	13 (43)	0.866
HCV RNA positive (n = 14)	9 (27)	5 (17)	0.312
TTV and HCV RNA positive (n = 2)	2 (6)	0 (0)	0.129

Abbreviations: TTV = torque teno virus; HCV = hepatitis C virus; SENV = SEN virus.

subtyping SENV infection in serum was established. 103 serum samples were analyzed, including 63 from long-term hemodialysis patients; patients positive for HBV were excluded because of the effect on liver function. Patients with HCV were not excluded due to the high prevalence of HCV in Egypt, and the need to study its relationship to SENV infection. Forty serum samples were included in the study as a control group; 20 samples were from patients with CKD who not yet started hemodialysis, to investigate the role of dialysis as a probable transmission route for SENV infection, and the other 20 were from apparently healthy blood donors who agreed to their samples being investigated.

The study found a high prevalence of SENV of 52.4% in hemodialysis patients compared with only 10.0% in the control group. The prevalence of SENV in this study is higher than that observed in Japanese hemodialysis patients (38.0%) [5] and Italian hemodialysis patients (40.9%) [12], and is similar to that observed in Slovakian hemodialysis patients (50.0%). In contrast to these results, other studies found no significant difference in the prevalence of SENV infection between the hemodialysis patients and healthy blood donors [13,14]. This suggests that SENV may be transmitted by routes other than the parenteral route [13], such as the feco-oral route. This

possibility is supported by the fact that the prevalence of SENV is higher in patients with acute hepatitis A infection, which is transmitted by the feco-oral route, than in healthy adults [1], and by the finding that TTV, which is distantly related to SENV, can be transmitted by the feco-oral route [5]. This study also found that the prevalence of SENV was high in patients with CKD who were not undergoing hemodialysis (50%), which confirms the involvement of routes other than dialysis for viral transmission, for example, invasive investigations and therapeutic procedures that lack preventive measures for blood borne infection. A high prevalence of SENV infection has been found among patients with renal disorders such as glomerulonephritis (40.0%) and diabetic nephropathy (21.4%) [12].

There is wide variation in the prevalence of SENV in the general population; with rates of 1.8% in the United States [4], 10% to 22% in Japan [15], 15% in Taiwan [16], and 5% in Thailand [17]. This could be attributed to the heterogenous distribution of SENV infection and its varied circulation within different geographical areas.

SENV-H was the genotype preferentially detected in the hemodialysis patients in this study (23/63; 36.5%), whereas SENV-D was found in only 9 of 63 hemodialysis patients (14.3%). These prevalences

are similar to those reported from Italy of 30.7% and 13.6% for SENV-H and SENV-D, respectively [14]. In contrast to these results, the distribution of SENV-D, SENV-H, and both SENV-D and -H among hemodialysis patients in Japan was 61%, 22%, and 17%, respectively. These prevalences do not differ significantly from the healthy controls (SENV-D, 77%; SENV-H, 15%; both SENV-D and -H, 8%) [p > 0.2] [5]. This may be due to different geographical distribution [2], or differences in the sensitivity of the genotyping assay due to different primers being used [8].

Surprisingly, in this study, 16% of hemodialysis patients were positive for SENV of non-H non-D type, but another variant of the 9 strains (A to I). These results need to be confirmed by sequencing analysis. However, Pirovano et al reported that SENV-B was detected in 15.7% of hemodialysis patients in their study [12].

In agreement with previous studies [5,8], there was no relationship between SENV infection and age, sex, ALT level, history of blood transfusion, and duration of dialysis in this study. However, 16% of the patients in this study had undergone surgical procedures, 60% of whom were found to have SENV infection. This result agrees with those reported from Slovakia, indicating that previous surgery is an important risk factor for SENV transmission [6]. Therefore, surgical procedures, environmental blood contamination, and hospital exposure via health care personnel could be important routes of transmission of SENV.

Until recently, there has been no evidence that SENV infection either causes hepatitis or worsens the course of coexistent liver disease, including infections with hepatitis A virus, HBV, or HCV [2]. In this study, there was no significant difference in the frequency of SENV infection between hemodialysis patients with positive HCV antibody (15/33; 45%) and those with negative HCV antibody (18/33; 54%).

In this study, abnormal ALT levels were uncommon in hemodialysis patients infected with SENV alone or in patients coinfected with HCV RNA (34.6 ± 12.0 and 33.1 ± 6.0, respectively). However, ALT levels were significantly higher in patients who were only infected with HCV RNA (49.3 ± 1.2 IU/L). However, the clinical relevance of SENV infection in association with hemodialysis and its association with liver disease remains uncertain, as SENV infections are highly dynamic during both viral clearance and reinfection [12]. Also, there is no antibody assay to test for acute or chronic infection, and patients who are negative for SENV could have previously had the infection and recovered, and would therefore have antibodies [18]. Further studies are needed to ascertain the association of SENV with liver disease [12].

Regarding coinfection with HCV and TTV, this study found that the distribution of HCV-RNA and TTV-DNA did not differ significantly between SENV-infected and non-infected hemodialysis patients (HCV RNA: 9/33 [27%] vs 5/30 [16.6%]; TTV-DNA: 15/33 [45%] vs 8/30 [26%]). This non-significant rate of TTV among SENV-infected patients, which was also reported by Kobayashi et al [5], suggests that SENV infects humans independently of the occurrence of TTV infection.

Further analysis of the 28 hemodialysis patients with HCV antibody resulted in a high but non-significant prevalence of SENV infection among patients who have recovered from HCV infection (11/18; 61%) compared with HCV viremic patients (5/10; 50%). This finding was also observed by Umemura et al [19], who reported that HCV was less prevalent among patients with SENV-H viremia (14%) than among patients without SENV-H viremia (34%) [p = 0.033] in an area of high HCV endemicity [19]. This result suggests a possible protective role of SENV against HCV or assistance with HCV clearance, making SENV worthy of further study.

In conclusion, SENV-H was the genotype preferentially detected in hemodialysis patients in this study. The presence of SENV alone or in patients coinfected with HCV did not induce alterations in ALT levels. However, further research is necessary to clarify whether there might be a significant change in liver function during certain periods of SENV infection. The reduced prevalence of active HCV infection among patients with SENV viremia compared with those without SENV viremia should invite further study into a possible protective role of SENV against HCV infection.

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