

# Molecular epidemiology of SEN virus among blood donors and renal dialysis patients

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**Abstract.** *Background and purpose:* The SEN virus (SEN-V) is a single-stranded circular, non-enveloped DNA virus that has been linked to blood transfusion and is thought to be a major cause of post-transfusion hepatitis. The two SENV types, SENV-H and SENV-D, are non-A to E hepatitis viruses in those who are infected. The purpose of this study is to find out how common SENV and its variations are among renal dialysis patients and healthy blood donors. *Methods:* The study used a cross-sectional design, with 300 blood samples collected from KFMMC patients, 150 from healthy blood donors and 150 from renal dialysis patients, between January 2019 and January 2021. The samples were screened for the presence of SENV-D and SENV-H. using nested PCR. *Results:* Molecular analysis of the SEN virus revealed that 9.3% of the samples (14 out of 150) tested positive for SEN virus infection in renal dialysis patients. The data from healthy donors revealed that 10% of the samples tested positive for the SEN virus (15 out of 150). *Conclusions:* The presence of SEN-V in healthy blood donors and renal dialysis patients demonstrates the virus's blood-borne nature and emphasizes the dangers of blood-borne transmission. ([www.actabiomedica.it](http://www.actabiomedica.it))

**Key words:** SEN-V, SENV-D, SENV-H, dialysis, blood, blood donor, RNA virus.

## Introduction

SEN virus (SEN-V) is a non-enveloped, blood-borne virus with a single-stranded circular DNA genome. Phylogenetic analysis demonstrates that there are nine different genotypes for this virus (1). SEN-V could be related to post-transfusion hepatitis, and infections with this virus in blood donors and hepatitis patients differ markedly by geographic region (2). SEN-V belongs to the Circoviridae family, Circovirus genus, and it contains approximately 3800 nucleotides (3,4).

Nine different genetic variants have been identified of SEN-V (A to I), which vary by at least 25% in their nucleotide series (5,6). The most predominant SEN-V genotypes are SEN virus D (SEN-D) and

SEN virus H (SEN-H), which are usually discovered in patients with hepatitis (non-A-E hepatitis), and less common in healthy blood donors (1,5). SEN-V infection can be transferred from person to person through different means, including drug addict by injection reuses, blood transfusion, organ, or haematological progenitor cells transplantation, and from mother to fetus (4,7).

SEN-V infections have been considered a major risk factor for dialysis patients. Various studies have reported the high prevalence of SEN-V virus in dialysis patients and blood donors with hepatitis in different world regions (8,9). SEN-V-D and SEN-V-H have been reported to be linked with post-transfusion hepatitis (10). In a study, Rizvi et al. reported

that SENV-D genotype was detected in 38% and SENV-H genotype in 58% cases of hepatitis patients (11). Mohamed et al. found that the two genotypes (D and H) of SEN virus were prevalent in blood transfused patients and renal dialysis patients, which they characterized as a high-risk group for SEN virus (12).

The prevalence of SEN-V varies geographically among healthy blood donors in United States, Japan, Taiwan, Thailand and Germany with 1.8%, 10 to 22%, 15.5% and 8 to 17%, respectively. While some studies suggest that about 30% recipients of postoperative transfusion are positive for SEN-V compared to only 3% positive patients who did not receive a blood transfusion in postoperative procedures (13). A major correlation has been found between the volume of blood used for transfusion and the risk of developing SEN-V infection, suggesting that the viral load is a major determinant of infection (14). Moreover, the transmission of the SEN virus through transfusion has been confirmed by the presence of more than 99% similarity between SEN virus variants in donor and recipient blood sera (15).

Co-infections or simultaneous infections with SEN-V and hepatitis virus and human immunodeficiency virus type 1 (HIV-1) have been recorded (16). These concurrent infections signify the blood-borne transmission of these viruses and SEN-V (1). SEN-V was discovered in the blood of posttransfusion hepatitis patients. However, it is confirmed that SEN-V is transmitted by blood transfusion, and further studies are required to understand its exact role in the future (17).

A study in Iran showed that patients on maintenance renal dialysis patients suffer from an increased risk of blood-borne viral infections, especially SEN-V infection. They reported that PCR results for SEN-V-D/H DNA indicated that 28.63% of patients were positive for SEN-V-D, and 14.53% were positive for SEN-V-H. Moreover, a co-infection SEN-V-D/H was found in 9.69% of 227 patients. The study concluded that the prevalence of SEN-V-D/H renal dialysis patients is higher as compared to healthy blood donors (9). Similarly, data suggests that SENV-D and -H are present in around 2% of US donors, transmitted by blood transfusions (18). A strong association of SEN-V with transfusion-associated non-A to

E hepatitis has been found, but most SEN-V-infected recipients did not develop hepatitis (15).

Recently, one study about the SEN virus in Saudi Arabia among healthy blood donors shown that 10.8% of them positive with SEN virus (19). The present study will use the molecular technique for SEN virus presence among healthy individuals compared to hemodialysis patients, using nested-polymerase chain reaction (Nested-PCR). The study's importance is to improve public confidence regarding blood transfusion safety and minimize the transmission risk of the virus through blood donations to get "zero risk" blood. One remarkable progress in transfusion safety is implementing more sensitive viral nucleic acid screening assays that have nearly eliminated HIV and hepatitis viruses (HBV & HCV) by blood transfusion.

## Materials and methods

### *Study design and samples collection*

This cross-sectional design and analytical study were included 150 healthy blood donors and 150 renal dialysis patients from tertiary care military hospital in Saudi Arabia, the King Fahd Military Medical Complex (KFMMC). Patient information was anonymized, and the samples collection extended from January 2019 to January 2021. HBsAg, anti-HCV, and anti-HIV antibodies were all negative in all samples. Blood samples were collected into sterile gel tubes without anticoagulant from each participant. The serum was then centrifuged at 3000 rpm, aliquoted, and stored at -80 °C until used. The Institutional Review Board (IRB) of the Prince Sultan Military College of Health Sciences, Dhahran, Saudi Arabia, approved this study (IRB-2018-CLS-004).

### *DNA extraction and SEN virus detection by nested PCR*

A DNA Isolation Mini-Kit (Norgen Biotek Corp, Ontario, Canada. Cat. # 46380) was used to extract DNA from 200µl of serum. The extracted DNA was subsequently stored at -80°C. The DNA of the SEN virus was amplified by using modified and optimizing

nested polymerase chain reaction (PCR) protocol from previous studies (6,20). In the first round, A 25 µl of PCR mixture containing 12.5 µl of an Absolute Master Mix (MOLECULE), with 1 µl of each of the forward and reverse primers of 10 µM/µl (SEN-V AI-1F and SEN-V AI-1R), 5 µl of the extracted DNA template, and finally 5.5 µl of Nuclease-free sterile water. The two primers were used to amplify a conserved region for all SENV genotypes (A-I) that consist of 349-bp, forward primer AI-1F (5'-TWCYMAACGAC-CAGCTAGACCT-3') and reverse primer AI-1R (5'-GTTTGTGGTGAGCAGAA CGGA-3') (6). The PCR program comprised of 35 cycles: 94°C for 5 minutes; 94°C for 1 minute, 53°C for 1 minute, and 72°C for 1 minute, followed by the extension reaction at 72°C for 10min in a thermal cycler (Bio-Rad, USA).

In the second round, specific primers were used to amplify of the SEN-V D, and SEN-V H. These primers were forward and reverse primers for the SEN-V D, which included D-1148F (5'-CTAAGCAGCCCTAACACTCATCCAG-3') and D1341R (5'-GCAGTTGACCGCAAAGTTACAAGAG-3'). Forward and reverse primers for SEN-V H had H-1020F (5'-TTTGGCTGCACCTTCTGGTT-3') and H-1138R (5'-AGAAATGATGGGTGAGT-GTTAGGG-3'). 25 µl PCR mixture was prepared with the 5 µl from the first-round product as a DNA template. The PCR program comprised of 30 cycles: 94°C for 5 minutes; 94°C for 1 minute, 54°C for 1 minute, and 72°C for 1 minute, with a final extension at 72°C for 10 minutes.

DNA samples considered positive after 2% agarose gel electrophoresis was also carried out and samples with 195-bp and 119-bp DNA segments indicated the presence of SENV-D and H genotypes, respectively.

## Results

### *Prevalence of SEN-V in hemodialysis patients*

The molecular screening of hemodialysis patients for the presence of SEN-V showed that 9.3% of patients on renal dialysis patients were found to be positive for SEN-V. Among the SEN-V positive patients, the prevalence of virus in males was 50%, and it was 50% in females. The age-wise analysis of respective data showed that the highest number of SEN-V positive cases in both genders were present in the age group 30-49 years. The lowest number of infected patients belonged to age groups 14-29 and 70-79. Moreover, the positive samples were analyzed for the presence of SEN-V variants, which showed that 93% were SENV-H, 7% were found to have co-infection of both SENV-H and SENV-D (Table 1).

### *Prevalence of SEN-V in blood donors*

The data from healthy donor showed a SEN virus positivity in 10% of the samples, with a prevalence percentage of 93% SENV-H and 7% SENV-D in positive samples. The age-wise analysis of respective data showed that the highest number of SENV positive cases in men was present in the age group (30-49) years old. In women, the only case reported belonged to the age group 15-29 years. Males were found to be more affected by SEN-V, as 93.3% of men were found to carry the viral DNA (Table 2), and the rest were females (6.7%).

## Discussion

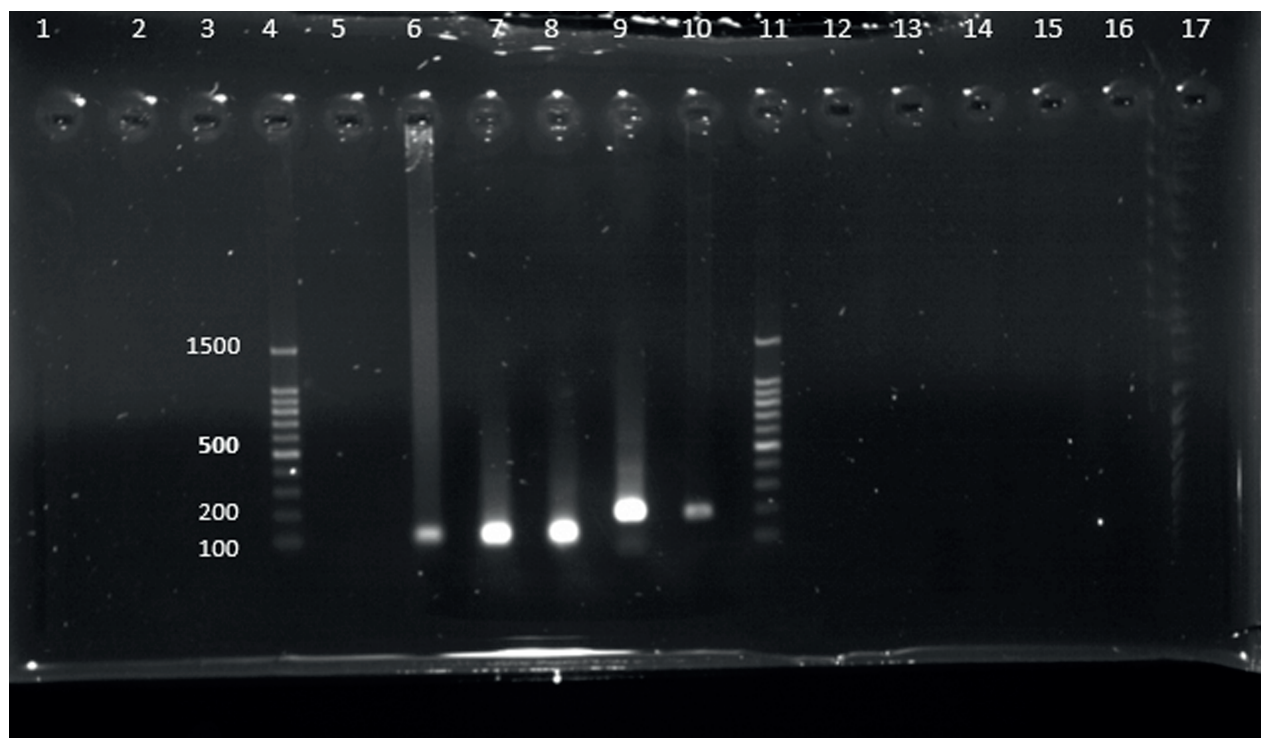
The SEN virus (SEN-V) belongs to a group of single-stranded circular, non-enveloped DNA viruses

**Table 1.** Prevalence of SEN-V and SENV variants in hemodialysis patients.

Age group	Male (92)				Female (58)			
	14-29	30-49	50-69	70+	14-29	30-49	50-59	70+
SEN-V DNA	1	4	2	0	1	3	2	1
SENV-H	1	3	2	0	1	3	2	1
SENV-D	0	0	0	0	0	0	0	0
Co-Inf (SEN-D & SEN-H)	0	1	0	0	0	0	0	0

**Table 2.** Prevalence of SEN-V and SENV variants in healthy donors.

Age Groups	Male (119)			Female (31)		
	15-29	30-49	50-69	15-29	30-49	50-69
SENV	5	7	2	1	0	0
SENV-H	5	6	2	1	0	0
SENV-D	0	1	0	0	0	0
Co-Inf(SENV-D & SENV-H)	0	0	0	0	0	0

**Figure 1.** Electrophoresis of the nested PCR Product. Lane (4,11) ladder (100 base pairs); Lane 5, negative control; Lane 6, SENV-H positive control; Lane 10, SENV-D positive control; Lane 1, 2, 3,5,12,13,14,15,16 and 17 negative samples for both SENV-D and H; Lane 7 and 8 SENV-H positive sample; and Lane 9, SENV-D positive sample.

that have recently been with renal dialysis patients and blood donors (13). In recent years, the SEN virus has been recognized to have a certain role in hepatic pathogenicity. SEN virus and its variants (SENV-D, SENV-H) categorized as a possible etiological agent for the transmission of hepatitis and is linked with various high-risk groups such as dialysis patients and low-risk groups such as blood donors (19,21). In the present study, 300 samples for renal dialysis patients and healthy blood donors were analyzed for SEN virus presence. The results showed that 9.3% of patients

on renal dialysis were positive for SEN-V, with the highest number of cases in the age group 30-49 in both genders. Among the SEN-V positive patients, the prevalence of virus in males was 50%, and it was 50% in females. Moreover, the SEN-V positive samples were analyzed for the presence of SENV variants, which showed that 93% of the positive samples were SENV-H, 7% were found to have co-infection of both SENV-H and SENV-D (Table 1).

The present study also highlights the prevalence of the SEN virus in healthy blood donors as the major

mode of transmission is blood transfer between individuals. The present data from healthy donors showed a SEN virus positivity in 10% of the samples, with a prevalence of 93% SENV-H and 7% SENV-D in positive samples. Males were found to be more affected by SEN-V, as 93.3% of men were found to carry the viral DNA (Table 1). Similar findings have been reported in a study in Iran, where a prevalence of 90.8% of SEN-V infection was found in healthy blood donors. They suggested that healthy blood donors infected by SEN-V play an essential role in the transmission of SENV, especially in thalassemic patients who receive frequent blood transfusions (1). Also, this finding similar to the study reported by Elmoeiz et al. in 2021, who reported that the prevalence of SEN virus among healthy donors was 10% in the Saudi population (19). Those healthy donors infected by the SEN virus have an etiological agent for transmitting hepatitis and is linked with various high-risk groups such as dialysis patients.

In contrast, one study has been reported by Kobayashi et al., who reported that SEN-V infection was significantly ( $P=0.012$ ) more common in renal dialysis patients (38%) as compared to the controls (22%) (8). They also found that SEN-V infection was not associated with the amount of transfusion or duration of hemodialysis. This study includes only 91 renal dialysis patients comparing to 51 non-dialysis subjects with chronic kidney diseases but without blood transfusion (8). A recent study analyzed the prevalence of SEN virus in renal dialysis patients using nested PCR and reported that 4% were positive for SEN virus. According to their study, the risk factors associated with the prevalence of SEN-V included frequent hospitalization, multiple blood transfusion episodes and hemodialysis (22). In another study, the prevalence of SENV-H was higher in renal dialysis patients than the variant SENV-D. Abd El-Hady et al. detected SEN virus in 89.1% (49 out of 55) renal dialysis patients versus 16% (4 out of 25) controls. The SENV-H was higher prevalence than SENV-D among both cases and controls groups (23).

## Conclusion

The high prevalence of SEN virus in healthy blood donors and renal dialysis patients highlights the

importance of proper blood screening measures along with HIV, Hepatitis B, and Hepatitis C. SEN virus is a blood-borne virus. It can be transmitted from infected individual to blood recipient and cause non-A-E hepatitis. Moreover, the variants of SEN virus, SENV-D and SENV-H have been found with renal dialysis patients and blood donors. Further studies need to be carried out on the pathogenesis of SENV, especially its effect on the liver and kidneys.

**Data Availability:** The datasets analyzed during the current study are available from the corresponding author on reasonable request.

**Ethical Approval:** The Institutional Review Board (IRB) of the Prince Sultan Military College of Health Sciences, Dhahran, Saudi Arabia, approved this study (IRB-2018-CLS-004).

**Funding:** This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

**Acknowledgements:** The authors would like to thank KFMMC for providing us with the patient samples. The authors would like to extend their thanks to Anisah Amer, Aljohara Almulhem, Nouf Almousa and Nourah Aldossary for helping us in sample collection.

**Conflicts of Interest:** Each author declares that he or she has no commercial associations (e.g., consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article.

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Received: 8 March 2022

Accepted: 10 April 2022

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