The Prevalence of SEN Virus Infection in Blood Donors in Iran

Zohreh Sharifi PhD*, Mahmood Mahmoodian-Shooshtari PhD*, Ali Talebian MD*

Background: SEN virus is a blood-borne, single-stranded, nonenveloped DNA virus. Two of its strains (D and H), appear to be associated with non-A-to-E hepatitis more frequently than the others, although it is not clear whether this observation has any significance. The prevalence of SEN virus in otherwise healthy individuals, including blood donors, differs markedly by geographic region. In this study, an investigation to evaluate the prevalence of SEN virus strains among blood donors in Tehran was carried out.

Methods: Sera of 260 blood donors who were negative for hepatitis B surface antigen (HBsAg) and third-generation hepatitis C virus antibody (anti-HCV) were tested for SEN virus-D and -H DNA. DNA was extracted from plasma of 260 blood donors and amplified by semi-nested polymerase chain reaction.

Results: SEN virus-D viremia was detected in four (1.5%) of the 260 blood donors (95% confidence interval (CI), 0.0 – 3%). SEN virus-H viremia was detected in 47 (18.08 %) of the 260 blood donors (95% CI, 13.4 – 22.8%). Both SEN virus-D and SEN virus-H viremia were detected in nine (3.4%) of the 260 blood donors (95% CI, 1.2 – 5.7%). SEN virus-D or SEN virus-H viremia was identified in 60 (23.08%) of the 260 blood donors (95% CI, 18.08 – 28.08%).

Conclusion: Out of the 260 blood donors, 60 (23%) were infected by SEN virus-D/H. The prevalence of SEN virus-H is more than SEN virus-D. Our results also showed that the high prevalence of SEN virus in healthy blood donors with no history of blood transfusion may attribute to the transmission modes other than parenteral transmission.

Introduction

Recently, another novel virus, called SEN virus (SENV), was described. SENV is a single-stranded circular virus of approximately 3,900 nucleotides. By phylogenetic analysis, eight different strains of SENV have been identified. SENV is a member of the Circoviridae family, a group of small, single-stranded, non-enveloped circular DNA virus that includes TT virus (TTV), TUS01, SANBAN, and YONBAN. The characteristic parallel with the TTV is that the viruses may share a common ancestor.

SENV is transmitted by blood, as demonstrated by comparing the sequence homology between donors and recipients. Moreover, transfused patients are at higher risk of acquiring SENV than nontransfused patients. Risk of infection in transfused patients increased proportionally with the number of units of blood transfused.

SENV is a DNA virus that was discovered in the serum of an injection drug user (IDU) infected by human immunodeficiency virus (HIV). This virus was found subsequently in a large percentage of IDUs and polytransfused patients.

Although SENV has been observed in patients with acute and chronic liver disease of unknown etiology, its role in the pathogenesis of liver disease is not yet known. The prevalence of SENV did not significantly differ between patients with acute or chronic hepatitis and those with non-viral liver disease. There was no significant difference between the rates of SENV-positive patients with acute hepatitis and blood donors. SENV was detected at almost the same frequency
in patients with and without liver disease. SENV does not seem to contribute either to the pathogenesis of liver disease or to the development of hepatocellular carcinoma from chronic liver disease. It is not known whether SENV may be a causative agent in some cryptogenic hepatitis.

In this study, an investigation to evaluate the prevalence of SENV strains among blood donors in Iran was carried out.

Materials and Methods

Serum samples
Two hundred sixty blood samples were obtained from blood donors. All the blood donors were healthy volunteers who met all standard eligibility criteria for donation (i.e., negative test results for hepatitis B surface antigen (HBsAg) and for antibodies to HCV and HIV). Two milliliters of blood was taken from each donor and then centrifuged at 3500 g at room temperature. Sera were separated and transferred into fresh tubes. The serum samples were kept at -70°C until the day of examination. All samples were tested for SENV-D and SENV-H DNA by polymerase chain reaction (PCR) during May 2006 through September 2006.

This study was reviewed and approved by the Medical Ethics Committee of Research Center of Iranian Blood Transfusion Organization (IBTO). A written informed consent was taken from healthy blood donors prior to enrollment into the study.

Detection of SENV DNA by PCR
The presence of SENV-D and SENV-H DNA was determined by genotype-specific PCR with specific primers. DNA was extracted by high pure viral nucleic acid kit (Roche Diagnostics, Germany) according to manufacturer’s instruction and DNA was stored in -70°C.

Total DNA was extracted from 200 µL serum and resuspended in 50 µL elution buffer. The oligonucleotide primers were synthesized according to the published SENV sequences. For the PCR, 20 µL reaction mixture containing 2µL of the DNA sample, 1X PCR buffer (10 mM Tris-HCL [pH 9.0], 50 mM KCL, 1.5 mM MgCL2, 0.01% gelatin, and 0.1% Triton X-100), 200 µM of each dNTP, 0.2 µM of each primer (sense primer for SENV, 5'-CC[C/G] AAA CTG TTT GAA GAC [C/A] A -3', antisense primer, 5'- CCTCGG TT[G/T] [C/G]AA A[G/T] G T[C/T] T GAT AGT-3', and 1U of Taq DNA polymerase was amplified in a thermal cycler.

The reactions consisted of preheating at 94°C for three min, 30 cycles of denaturation at 94°C for one min, annealing at 50°C for one min, extension at 72°C for 40 s, and final incubation at 72°C for five min. The second PCR step was carried out with a 20- µL PCR reaction mixture containing 2 µL of the first-step amplification product, the same PCR buffer used for the first PCR step, 0.2 µM of each primer (sense primer for SENV-D,5'-GTA ACT TTG CGG TCA ACT GCC CCT [A/T]GT [C/T]AG TTG GCG GTT-3'; universal antisense primer, 5’-CCTCGG TT[G/T] [C/G]AA A[G/T] GG T[C/T]T GAT AGT-3', 200 µM of each dNTP, and 1U of Taq DNA polymerase. PCR consisted of denaturation at 94°C for one min, annealing at 58°C for one min, extension at 72°C for 40 s, and final incubation at 72°C for five min. The amplification products were separated by 2% agarose gel electrophoresis and stained with ethidium bromide and photographed under ultraviolet light.

Nucleotide sequencing was carried out with an automatic DNA sequencer (ABI model 373; Applied Biosystems) and performed in both orientations for confirmation. Nucleotide sequence homologies between these isolates and the published reference sequence were determined using BLAST search.

Results

PCR products were detected by 20 g/L agarose gel. The results showed that there was a 230-bp band in the gel (Figure 1).

SENV-D viremia was detected in four (1.5%) of the 260 blood donors (95% confidence interval (CI), 0.0 – 3%). SENV-H viremia was detected in 47 (18%) of the 260 blood donors (95% CI, 13.4 – 22.8%). Both SENV-D and SENV-H viremia were detected in nine (3.4%) of the 260 blood donors (95% CI, 1.2 – 5.7%). SENV-D or SENV-H viremia was identified in 60 (23%) of the 260 blood donors (95% CI, 18 – 28%). The prevalence of SENV-H was 12 folds higher than that of SENV-D in healthy blood donors.

Twenty- five PCR fragments of SENV-D and SENV-H were randomly chosen and sequenced. The nucleotide sequences from 25 PCR products were 89 – 98% identical among SENV-D isolates.
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(GenBank accession numbers [AY052805 and AY052806]). The nucleotide sequences from 25 PCR products were 87 – 95% identical among SENV-H isolates (GenBank accession numbers [AY052812 and AY052820]).

Discussion

SENV consists of eight strains from A to H. Two of the strains, D and H, appear to be associated with non-A-to-E hepatitis more frequently than the other strains, although it is not clear whether this observation has any significance.

Evidence to support transmission of SENV by blood transfusion has been reported. SENV DNA was detected in 86 out of 286 (30%) previously uninfected patients who received transfusion after surgery, compared with three out of 97 (3%) patients who did not receive blood transfusion. Strains D and H have been found in 30% of cases of transfusion-associated non A-to-E hepatitis in the United States, compared with 1.8% of healthy blood donors.

The distribution of SENV strains D and H among blood donors or other healthy populations varies geographically. Most studies, however, have not evaluated other strains, but rather focused only on strains D and H because of their possible but unproven association with post-transfusion hepatitis. The prevalence of SENV isolated from serum samples of otherwise healthy persons has been reported to be 1.8% in the United States, 10 – 22% in Japan, 15 – 51% in Taiwan, 8 – 17% in Germany, 5% in Thailand, 5% – 24% in Greece, and at least 13% in Italy. The results of this study showed that SENV-D/H is detectable in 23% of blood donors in Iran. This percentage is similar to that reported for donors in Greece (24%), in China (31%), and in Germany (17%) but is higher than that reported for donors in the United States (1.8 %), in Thailand (5%), and in Italy (13%).

Primi and Scottini found that SENV and transfusion transmitted virus (TTV) had similar structure. SENV is distantly related to TTV with which it shares a similar structural organization but has only about 55% nucleotide sequence homology and about 37% amino acid homology. TTV is widespread worldwide, as revealed by the prevalence of TTV infection rates among blood donors ranging from 34% in the United States to 98% and 100% in Singapore and Saudi Arabia, respectively. The prevalence of TTV infection among a group of Iranian blood donors was 20 – 41%. These findings led to the suggestion that TTV might be an example of a highly successful and widely distributed virus capable of establishing a commensal relationship with its host. TTV was shown to be excreted with feces and its transmission by this route has been suggested. Transmission of SENV from infected mother to their newborn infants has been occurred. This is a characteristic that SENV shares with some other blood borne viruses such as hepatitis B virus.

Schroter et al recently reported that because of the high prevalence of SENV-H among blood donors (16.8%) and the nearly identical prevalence among patients on maintenance hemodialysis (12.8%), significant modes of transmission apart from the parenteral route might be important for SENV-H spreading as well. As suggested for TTV, SENV might be another example of a highly successful and widely distributed virus capable of establishing a commensal relationship with its host without causing clinical disease.

On the basis of the results of this study, the high rate of detection of SENV DNA in the sera of the healthy blood donors may indicate a high prevalence of SENV. Therefore, it can be argued that SENV might also be transmitted by additional routes. Although the SENV family only seems to be distantly related to TTV, SENV could be

Figure 1. Electrophoresis of PCR products. Lane 1 negative control; Lane 2 marker (100bp); Lanes 3, 4 SENV-D; Lanes 5, 6 SENV-H; and Lanes 7, 8 positive control.
another example for a human commensal virus.

In conclusion, the high prevalence of SENV in healthy blood donors with no history of blood transfusion may attribute to the transmission modes other than parenteral transmission.

References


