



RAPID COMMUNICATION

Prevalence and clinical significance of SEN virus infection in patients with non A-E hepatitis and volunteer blood donors in Shanghai

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Abstract

AIM: To explore the prevalence of SEN virus (SENV) in patients with non A-E hepatitis and volunteer blood donors in Shanghai.

METHODS: According to the published gene sequences, primers from the conserved region were designed. Then, the prevalence of SEN virus in 30 samples from healthy voluntary blood donors and 30 samples from patients with non A-E hepatitis were detected by nested-PCR of SENV-D/H. Some PCR products were cloned and sequenced.

RESULTS: The specificity of genotype-specific PCR was confirmed by sequencing, the SENV DNA was detected in 53.3% of the patients with non A-E hepatitis and 10% of the blood donors. The prevalence of SENV-D/H viremia was significantly higher in patients with non A-E hepatitis than in blood donors ($P = 0.0002$). SENV-H subtype and SENV-D subtype were found in 2 and 1 samples, respectively from blood donors. SENV-H subtype, SENV D subtype, mixed SENV-D and SENV-H subtype were found in 8, 6 and 2 samples, respectively, from patients with non A-E hepatitis.

CONCLUSION: The gene type of SENV in patients with non A-E hepatitis and blood donors in Shanghai is D or H subtype, and transfusion is not the only transmitting form of SENV.

INTRODUCTION

A DNA virus-designated SEN virus (SENV), which was discovered in the serum of a human immunodeficiency virus type 1 (HIV-1)-infected patient, has been described recently^[1]. It is assumed to be transmitted parenterally and to cause posttransfusion hepatitis in humans. SENV is described as a small single-stranded, non-enveloped circular DNA virus containing a genome of approximately 3800 nucleotides, possibly belonging to the circoviridae family. To date, eight distinct strains of SENV (A-H) have been identified^[2]. There are 15%-50% sequence diversities among them. Phylogenetically, SENV is distantly related to TTV with which it shares a similar structural organization but only about 40%-60% sequence homologies^[3]. According to previous studies, the prevalence of these eight different strains of SENV (A-H) is different in each infected group. The prevalence of SENV-D or SENV-H strains was 2.25% and 92.31% in different groups including healthy blood donors, patients with acute or chronic non A-E hepatitis, respectively, suggesting that SENV-D or SENV-H is significantly associated with the pathogenesis of non A-E hepatitis^[3].

To date, the prevalence of SENV in patients with various forms of liver disease has been reported in many countries and several districts of China, but the results are not consistent to a certain extent, and the role

of SENV infection in patients with non A-E hepatitis or other viral hepatitis, and the transmitting form of SENV are not very clear^[4-7]. The purpose of this study was to investigate the prevalence and molecular biology characteristic of SENV in patients with non A-E hepatitis and volunteer blood donors in Shanghai, and the possible clinical significance of SENV infection in patients with non A-E hepatitis.

MATERIALS AND METHODS

Patients

A total of 30 serum samples from volunteer blood donors and 30 serum samples from patients with non A-E hepatitis from January 2005 to June 2007 in sixth people's Hospital affiliated to Shanghai Jiaotong University were studied. These cases included 42 men and 18 women, with a mean age of 37.2 ± 7.1 years (range, 19-65 years). All the serum samples were negative for both HBsAg and anti-HCV. The patients with non A-E hepatitis were defined as negative control for known serologic markers, including IgM anti-HAV, IgM antibody to hepatitis B core antigen (anti-HBc), hepatitis B surface antigen (HBsAg), HBV DNA, and antibodies to HCV, HDV and HEV. The patients had no history of HAV, HDV, HEV, EBV, CMV infection, or of adipositis hepatica and hepatic lesions induced by drugs, alcohol, cholestatic and autoimmunity, or of transfusion.

Detection of SENV DNA

The primers were designed according to the sequences submitted to GenBank with computer analysis to determine the outer primer by inner primer. All primers were synthesized by Shanghai Sangon Biological Engineering Technology & Services Co. Ltd. The sequences of primers are as follows (W = A or T, Y = C or T, M = A or C). SENV common primers: P1, 5'-TW CYCMAACGACCAGCTAGACCT-3'; P2, 5'-GTTTGTGGTGAGCAGAACGGA-3'. SENV-D primers: P3, 5'-CTAAGCAGCCCTAACACTCATCCAG-3'; P4, 5'-GCAGTTGACCGCAAAGTTACAAGAG-3'. SENV-H primers: P5, 5'-TTTGGCTGCACCTTCTGGTT-3'; P6, 5'-AGAAATGATGGGTGAGTGTAGGG-3'.

All blood samples were separated by centrifugation. The sera were stored at -80°C until SENV DNA analysis. Viral DNA was extracted from 200 µL serum with the QIAamp DNA blood mini kit (Qiagen) and resuspended in 100 µL elution buffer according to the manufacturer's instructions.

PCR mixture of 50 µL contained 0.5 µL (50 pmol/L) sense primer SENV-P2, 0.5 µL (50 pmol/L) antisense primer P1, 250 µmol/L of each dNTP, 6 µL DNA sample, and 2.5 U Taq DNA polymerase (TaKaRa). The reactions consisted of preheating at 94°C for 4 min, 35 cycles of denaturation at 94°C for 40 s, annealing at 55°C for 50 s, extension at 72°C for 50 s, and a final at 72°C for 10 min.

The second PCR step was carried out with 50 µL PCR reaction mixture containing 10 µL of the first-

step amplification product. The same PCR buffer was used for the first PCR step, 0.5 µL (50 pmol/L) sense primer P4 and antisense primer P3 for SENV-D, 0.5 µL (50 pmol/L) sense primer P6 and antisense primer P5 for SENV-H, 250 µmol/L of each dNTP, and 2.5 U Taq DNA polymerase. PCR consisted of preheating at 94°C for 30 s, annealing at 55°C for 50 s, extension at 72°C for 50 s, and a final incubation at 72°C for 10 min.

Determination of SENV genotypes

PCR amplified products were separated in 10 µL reaction mixture by electrophoresis on a 1.5% agarose gel, stained with ethidium bromide, and visualized using an ultraviolet transilluminator. Amplicons containing poly A tails and producing visible bands on agarose gel were excised from the gel and ligated to the pMD18-T vector (TaKaRa). DNA extracted from transformed *Escherichia Coli* was sequenced in Shanghai Sangon Biological Engineering Technology & Services Co, Ltd. The sequences excluding primer sequences were aligned with Cluster W to A-H SENV genotypes. The genotypes of SENV were determined by the phylogenetic trees.

Statistical analysis

Descriptive statistical data such as means and proportions were calculated. Frequency was compared between groups using the chi-square test or Fisher's exact test, and group means were compared using Student's *t* test. Stepwise logistic regression method was used to analyze the data. $P < 0.05$ was considered statistically significant.

RESULTS

Prevalence of SENV-D/H DNA in patients with non A-E hepatitis and blood donors

The nPCR results showed that 3 (10%) of the 30 volunteer blood donors and 16 (53.3%) of 30 patients with non A-E hepatitis were positive for SENV. Of 3 volunteer blood donors, 1 was positive for SENV-D and 2 were positive for SENV-H. Of the 16 patients with non A-E hepatitis, 6 were positive for SENV-D, 8 were positive for SENV-H, 2 were positive for both SENV-D and SENV-H DNA (SENV-D/H co-infection). The overall prevalence of SENV-D/H was 30%. After electrophoresis on 1.5% agarose gel stained with ethidium bromide on DNA product, the expected 349 bp, 118 bp and 193 bp bands were visualized on an UV transilluminator for SENV and SENV-D/-H, respectively (Figure 1). The positive rate of SENV infection was significantly higher in patients with non A-E hepatitis than in volunteer blood donors ($P = 0.0002$).

Sequencing PCR products and homology analysis

Randomly selected PCR products of P3 and P4 (118 bp) or P5 and P6 (193 bp) were sequenced. When the sequences of PCR positive products were compared with those of SENV by homology analysis, the

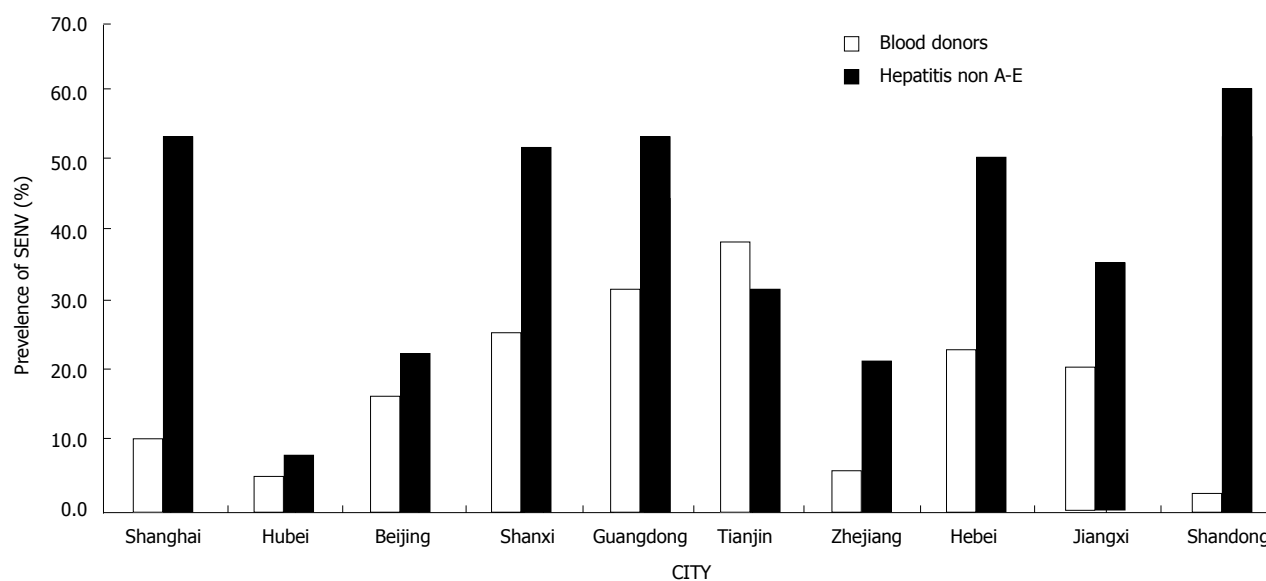


Figure 4 Prevalence of SENV in different provinces of China.

is 21% in patients with chronic hepatitis C^[25-27]. In this study, the detectable SENV DNA rate was 10% (3/30) in 30 volunteer blood donors and 53.3% (16/30) in 30 patients with non A-E hepatitis.

In the present study, SENV infection was found in blood donors and patients with non A-E hepatitis in Shanghai. We found that approximately 3.2%-38.9% of blood donors and 9.3%-59.6% of non A-E hepatitis had positive SENV (Figure 4) and the positive SENV DNA rate was significantly different in different areas, suggesting that we must continue the detection of SENV DNA prevalence in other provinces. Interestingly, the prevalence rate of SENV was higher in blood donors than in patients with non A-E hepatitis (38.9% *vs* 32.3%) in Tianjin.

Furthermore, SENV-H was found in 2 volunteer blood donors and SENV-D was found in 1 volunteer blood donor infected with SENV. SENV-H, SENV-D and mixed SENV-D/H were found in 8, 6, and 2 patients with hepatitis, indicating that the prevalence of SENV in Shanghai is relatively low. However, SENV infection was not associated with blood transfusion in 16 SENV positive non A-E hepatitis patients with no blood-transfusion history, indicating that blood transfusion transmission is not the only way for people to infect with SENV. Because of the limited number of serum samples in our study, we could not explain why SENV infection is related with blood transfusion or non A-E hepatitis.

In conclusion, SENV infection is higher in patients with non A-E hepatitis than in blood donors and the homology is 98%. Further study with a large number of samples is required to analyze the relationship between SENV infection and non A-E hepatitis.

COMMENTS

Background

SEN virus (SENV) is described as a small single-stranded, non-enveloped circular DNA virus, possibly belonging to the circoviridae family. To date, eight

distinct strains of SENV (A-H) have been identified. The prevalence of these eight different strains SENV (A-H) is different in each infected group. In the present study, the prevalence of SENV-D or SENV-H strain was 2.25% and 92.31% in blood donors and patients with acute or chronic non A-E hepatitis, suggesting that SENV-D or SENV-H is significantly associated with the pathogenesis of non A-E hepatitis.

Research frontiers

The prevalence of SENV in patients with various forms of liver disease has been reported in many countries and several regions of China, but the results are not consistent. The role of SENV infection in patients with non A-E hepatitis or other viral hepatitis and the transmitting form of SENV are not very clear. The clinical significance of SENV infection in patients with non A-E hepatitis is also not very clear.

Innovations and breakthroughs

SENV infection was studied in blood donors and patients with non A-E hepatitis in Shanghai. The results indicate that the prevalence of SENV in blood donors of Shanghai was relatively lower than that in other regions of China. SENV infection was not associated with blood transfusion in SENV positive patients with non A-E hepatitis with no blood-transfusion history, suggesting that blood transfusion transmission is not the only way to spread SENV.

Applications

The SENV infection rate is higher in non A-E hepatitis than in blood donors in Shanghai. Because of the limited number of serum samples in our study, we could not explain why SENV infection is associated with blood transfusion or non A-E hepatitis. Further study with a large number of samples is required to analyze the relationship between SENV infection and non A-E hepatitis.

Peer review

This paper describes the prevalence of SEN virus in blood donors and hepatitis patients. The study is well designed and provides important information about the prevalence of SENV in hepatitis patients.

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