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• CLINICAL RESEARCH •

Prevalence of a newly identified SEN virus in China

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Abstract

AIM: To establish nested-PCR methods for the detection of SENV-D and SENV-H and to investigate the epidemiology of SEN virus in China.

METHODS: According to published gene sequences, primers from the conserved region were designed. Then, 135 samples from healthy voluntary blood donors and 242 samples from patients with various forms of liver disease were detected by nested-PCR of SENV-D/H. Some PCR products were cloned and sequenced.

RESULTS: By sequencing, the specificity of genotype-specific PCR was confirmed. SENV-D/H DNA was detected in 31% of the blood donors, which was higher than those in America and Italy (2%), and in Japan and Taiwan (15-20%). The prevalence of SENV-D/H viremia was significantly higher in patients with hepatitis B and hepatitis C than in blood donors (59-85% *vs* 31%, $P<0.05$). The prevalence among patients with non-A-E hepatitis was significantly higher than among blood donors (68% *vs* 31%, $P<0.01$), and equivalent to that among patients with hepatitis B and C.

CONCLUSION: Nested-PCR with genotype-specific primers could serve as a useful SENV screening assay. SENV has the same transmission modes as HBV and HCV. The high prevalence in patients with non-A-E hepatitis may attribute to the transmission modes, and SENV may not serve as the causative agents.

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INTRODUCTION

Recently, a novel DNA virus designated SEN virus (SENV) was discovered in the serum of a human immunodeficiency virus type 1 (HIV-1)-infected patient[1]. Phylogenetic analysis[2] showed 8 strains (A-H) of SENV. To date, the ninth genotype (SENV-I) has been identified[3]. Among them, 2 SENV strains (SENV-D and SENV-H) were significantly associated with transfusion-associated non-A-E hepatitis[4]. SENV-D and SENV-H were also detected more frequently in patients with chronic liver disease and hepatocellular carcinoma (HCC) than in healthy adults[5,6]. However, the association of SENV infection with liver cell damage is far from clear, and further studies are needed to investigate the clinical relevance of SENV infection worldwide.

The purpose of this study was to develop a nested polymerase chain reaction (nPCR) method for the detection of SENV-D and SENV-H DNA in serum and elucidate its specificity. The presence of SENV-D and SENV-H viremia in patients with various forms of liver diseases and the possible role of SENV infection in non-A-E hepatitis were investigated.

MATERIALS AND METHODS

Patients

A total of 377 serum samples from March 1999 to April 2003 were studied in Xijing Hospital. They were divided into 5 groups as follows: 135 blood donors; 55 patients with acute hepatitis A virus (HAV); 126 with chronic hepatitis B; 20 with chronic hepatitis C; 41 with non-A-E hepatitis defined as negative control for known serologic markers, including I gM anti-HAV, I gM antibody to hepatitis B core antigen (anti-HBc), hepatitis B surface antigen (HBsAg), and antibodies to HCV, HDV and HEV.

Detection of SENV DNA

Viral DNA was extracted from 100 μL serum with the QIAamp blood kit (Qiagen) and resuspended in 20 μL elution buffer. PCR mixture of 20 μL contained 0.4 μmol/L sense primer SENV-P2 (5' -CC[C/G] AAA CTG TTT GAA GAC [C/A]A-3') (designed by ourselves), 0.4 μmol/L antisense primer Lucky2AS (5' -CCT CGG TT[G/T] [C/G]AA A[G/T]G T[C/T]T GAT AGT-3')[1], 200 μmol/L of each dNTP, 2 μL DNA sample, and 1 U *Taq* DNA polymerase. The reactions consisted of preheating at 94 °C for 3 min, 30 cycles of denaturation at 94 °C for 1 min, annealing at 50 °C for 1 min, extension at 72 °C for 40 s, and final incubation at 72 °C for 5 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, 0.4 μmol/L sense primer D10s (5' -GTA ACT TTG CGG TCA ACT GCC-3')[1] and antisense primer Lucky2AS for SENV-D; 0.4 μmol/L sense primer C5s (5' -GGT GCC CCT [A/T] GT [C/T]AG TTG GCG GTT-3') (designed by ourselves) and antisense primer Lucky2AS for SENV-H, 200 μmol/L of each dNTP, and 1 U *Taq* DNA polymerase, PCR consisted of preheating at 94 °C for 1 min, annealing at 58 °C for 1 min, extension at 72 °C for 40 s, and final incubation at 72 °C for 5 min.

Determination of SENV genotypes

Amplicons containing polyA tails and producing visible bands on agarose gel were excised from the gel and ligated to the pGEM vector. DNA extracted from transformed *Escherichia Coli* was sequenced. The sequences excluding primer sequences were aligned with Cluster W to all SENV genotypes, representative of TTV (TA278) and TLMV isolates. The genotype of SENV was determined by the phylogenetic trees.

Table 1 Prevalence of SENV infection in different hepatitis groups in China

Disease	*n*	Single SENV-D	Single SENV-H	Combined SENV-D/-H	Total *n* (%)
Acute hepatitis A	55	2 (3.6)	12 (21)	6 (11)	20 (36)
Chronic hepatitis B	126	17 (13)	16 (12)	41 (32)	74 (59)[b]
Chronic hepatitis C	20	4 (20)	4 (20)	9 (45)	17 (85)[b]
Acute+chronic non-A-E	41	10 (24)	7 (17)	11 (27)	28 (68)[b]
Blood donors	135	9 (6.7)	7 (5)	26 (19)	42 (31)

[b]$P<0.01$ *vs* Blood donors.

Statistical analysis

Data were analyzed by χ^2 test with Yates' correction or Fisher's exact test; $P<0.05$ was considered to be statistically significant.

RESULTS

Genotype-specific PCR with specific primers

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). The PCR products were inserted into pGEM-T vectors and sequenced. A phylogenetic tree of the SENV-D sequences (Figure 2A) from 8 blood donors (B7, B11, B13, B14, B28, B29, B31 and B33) and 7 patients with non-A-E hepatitis (N4, N9, N10, NC, NF, NG and NK) was constructed. To determine a root of the phylogenetic tree, TLMV was used as an out-group. SENV-D DNA sequences from study subjects clustered in a monophyletic group with the SENV-D prototype sequences. A phylogenetic tree of the SENV-H sequences (Figure 2B) from 7 blood donors (B1, B4, B11, B12, B28, B29 and B30) and 5 patients with non-A-E hepatitis (N4, N8, N10, NA and NF) was constructed. SENV-H DNA sequences from study subjects clustered in a monophyletic group with the SENV-H prototype sequences with the exception of 2 outliers (B1 and B28), which had closer homology to SENV-C.

The specificity of genotype-specific PCR was confirmed by sequencing. Here, all 15 patients diagnosed as SENV-D by the genotype-specific PCR had homology to SENV-D (Figure 2A), while 10 of 12 patients (83%) diagnosed as SENV-H by the genotype-specific PCR had homology to SENV-H, the other 2 had homology to SENV-C which was of the same genotype as SENV-H (Figure 2B).

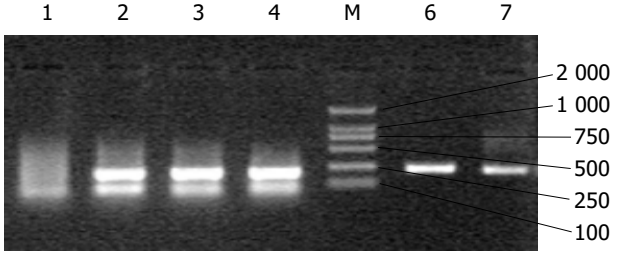

Figure 1 Electrophoresis of PCR products. Lane 1: Negative control; lane 2: Positive control; lanes 3, 4: SENV-D; lane 5: Marker DL2000; lanes 6, 7: SENV-H.

SENV prevalence in patients with liver diseases and in blood donors

SENV-D and/or SENV-H (SENV-D/H) DNAs were positive in 31% of healthy Chinese adults. SENV viremia was identified in 20 (36%) of 55 patients with acute or chronic hepatitis A, in 74 (59%) of 126 patients with chronic hepatitis B, in 17 (85%) of 20 patients with chronic hepatitis C, in 28 (68%) of 41 patients with acute or chronic non-A-E hepatitis. Compared with healthy blood donors, SENV infection was found more frequently in patients with hepatitis B, hepatitis C and non-A-E hepatitis (59-85% *vs* 31%, $P<0.01$, Table 1). However, the prevalence was comparable among patients with acute hepatitis A and healthy adults (31% *vs* 36%). The prevalence of SENV-H was 2-fold higher than that of SENV-D in patients with acute hepatitis A. Mixed SENV-D/H infection was common (Table 1).

DISCUSSION

Viral hepatitis is a worldwide disease[7] and imperils human health gravely. To date, 5 hepatitis viruses from HAV to HEV[8], which account for 8% to 90% hepatitis cases, have been discovered. But we could not find any hepatitis viruses in the left 10% to 20% cases that have typical viral hepatitis manifestations. Scientists speculated that the virus TTV was correlated with non-A-E hepatitis. Chinese scholars studied infection rate of TTV in different populations and its pathogenicity in liver[9-12], and found that TTV had no pathogenicity[13-15]. Afterwards, Primi *et al*.[1] found that SENV and TTV had similar structure. SENV has homogeneity of 55% at nucleotide level with TTV, but they only have homogeneity of 37% at amino acid level[2]. SENV appears to belong to a family of small, circular, non-enveloped, single-stranded DNA viruses that have been designated as circoviruses[16,17]. Phylogenetic analysis of SENV has shown the existence of 8 different variants (A-H). Among them, only SENV-D and SENV-H have higher prevalence ratios, and they are found in 2% of blood donors and >50% of persons with transfusion-associated non-A-E hepatitis in Italy and the United States. Although a strong association of SENV-D/H with transfusion-associated non-A-E hepatitis has been reported, whether SENV-D/H serves as a causative agent of post-transfusion hepatitis remains unknown[18-20].

The results of our present study indicate that nPCR amplified with genotype-specific primers could serve as a useful SENV screening assay. Moreover, because all components of the assay are readily available (specific antibodies are not required), viral testing can be performed in most laboratories that perform PCR. Our recent study indicated that 31% of healthy Chinese adults were positive for SENV-D/H DNA, a rate comparable to that of blood donors in Japan (10-20%)[5,21] and Taiwan (14-20%)[6] (age-specific prevalence), but higher than that of blood donors in the United States and Italy (2%)[22,23]. Co-infection with SENV has been frequently observed in 22-67% of patients with chronic hepatitis B[5,6] and 20-76% of patients with chronic hepatitis C[4-6,24,25]. Our study showed that the prevalence of SENV-D/H infection in patients with chronic hepatitis B and C were 59% and 85% respectively, implying that HCV, HBV and SENV may share common modes of transmission. Our study also indicated that compared with blood donors (31%), SENV-D/H infection was more frequent in patients with non-A-E hepatitis (68%), but comparable to those in patients with chronic hepatitis C and B (85% and 59%, respectively). These results can not support a causal role for SENV-D/H in the development of non-A-E hepatitis. Otherwise, we also documented that the prevalence of SENV-H was comparable to that of SENV-D in different subjects, such as blood donors, patients with chronic

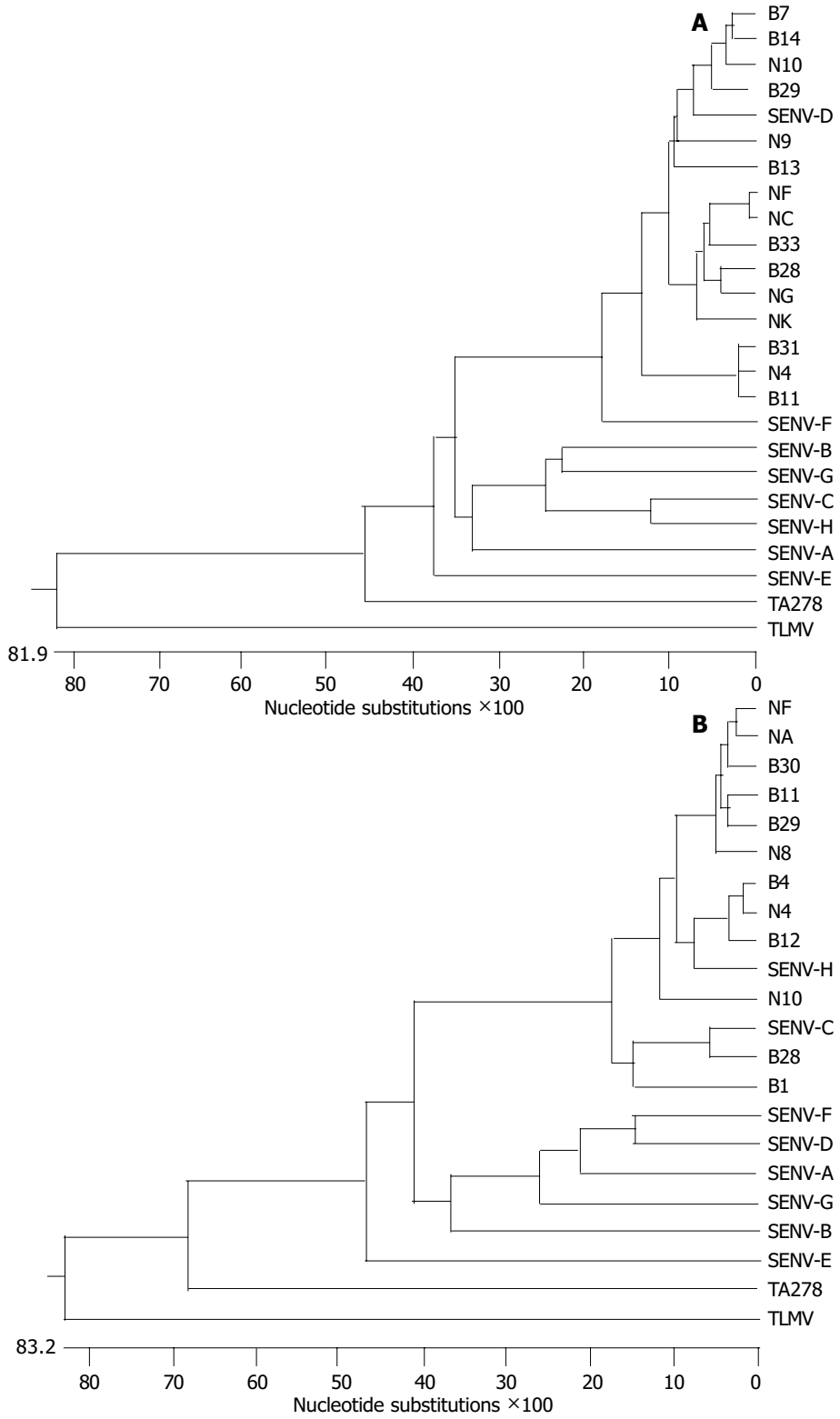

Figure 2 Phylogenetic tree of SENV by neighbor-joining method. A: The tree constructed on basis of 1 prototype TTV isolate (TA278), 1 TTV variant, 8 SEN virus isolates (A-H) and 15 SENV-D Chinese isolates. B: The tree constructed on basis of 1 prototype TTV isolate (TA278), 1 TTV variant, 8 SEN virus isolates (A-H) and 12 SENV-H Chinese isolates.

hepatitis B or C, and patients with non-A-E hepatitis, but among patients with acute hepatitis A, SENV-H was more prevalent than SENV-D ($P<0.05$). This phenomenon awaits further prospective studies.

In summary, the results of this study suggest that SENV has the same transmission modes as HBV and HCV[26]. The high prevalence in patients with non-A-E hepatitis may attribute to the transmission modes, and SENV may not serve as the causative agents. At present, SENV and the larger group of human circoviruses have no established pathogenicity, but they may have disease associations that have not been identified, or they may serve as commensal organisms that have some beneficial role in maintaining homeostasis in the host. They are worthy of more attention, even in the absence of confirmed disease associations.

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